An investigation of the potential of anaerobically digested piggery waste for use in food production, with particular reference to tomato and fish production.

Thesis

AN INVESTIGATION OF THE POTENTIAL OF ANAEROBICALLY DIGESTED PIGGERY WASTE FOR USE IN FOOD PRODUCTION, WITH PARTICULAR REFERENCE TO TOMATO AND FISH PRODUCTION.

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ABSTRACT.

The purpose of this study is to investigate the potential of animal waste for use in food production, by employing anaerobically digested piggery waste as a nutrient source for tomatoes and fish.

The problem has been configured in terms of a "Bioplex" (integrated agriculture) system, and as such has been tackled by employing a bastardised systems methodology, which seeks to recognise the relationships between the resource to be used, and the food production system itself. This assumption underlies the experimental work carried out in the thesis.

Initial experimentation comprised studies on the use of the piggery effluent in its raw form as a medium for the hydroponic production of tomatoes by nutrient film technique, and as a conventional fertiliser. These trials, and a study of the physical and chemical characteristics of the piggery waste indicated that in order to maximise the food production potential of the effluent, it should be viewed as a composite of three different fractions, each of which may be most usefully employed in a specific food production system.

The bulk of the thesis is concerned with the extraction of two distinct fractions of the effluent; the protein rich suspended solids, and the aqueous phase, by means of an oxidation technique.

The main criterion used to describe the success of the separation process is the application of the two products to food production operations, rather than the efficiency of the separation per se. To this end, the clarified liquid, and the separated solids were used as the basis of a nutrient solution for the hydroponic production of tomatoes, and a substitute protein source in the diet of common carp, respectively. The separation technique passed through two design iterations before products suitable as nutrient sources were generated.

As the trials on fish and tomatoes were closely linked to the separation technique, this work occupies the main body of the thesis, and the initial feasibility experimentation is presented in the form of appendices.

ACKNOWLEDGEMENTS.

Rather than let this section turn into the academic equivalent of an Oscar award speech, I would simply like to give my grateful thanks to the large number of people who have given me support, advice and assistance during the work on this thesis.

I must, however, formally thank my supervisor, Dick Morris, for his generosity of time and commitment to the project.

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An Explanation of the Thesis Structure, and a Guide to Information Abstraction.

Because of the methodological approach adopted in this work, the sequencing of experiments is non conventional. A diagrammatic representation of the "integrated agriculture" framework is given on page 5a. This thesis deals with selected problems within that system. Information relating to specific topics may be abstracted on a piecemeal basis by using the key below. Sequential reading will, however, give a more detailed understanding of the agricultural system under study.

Subject	Dealt with in Chapter/Appendix number												
	1	2	3	4	5	6	7	8	9	I	II	III	IV
Anaerobic Digestion	▨									▨			
Bioplex, Integrated Agriculture.	▨	▨											
Slurry as a Conventional Fertiliser.	▨	▨								▨	▨		
Hydroponics.		▨						▨				▨	
Animal Waste Separation Techniques.		▨	▨	▨						▨			
Animal Waste as a Substitute Protein.		▨			▨		▨						
Aeration of Slurry.			▨	▨		▨							
Fate of Copper in Piggery Waste.													▨

1. AGRICULTURE, ENERGY AND ANAEROBIC DIGESTION.

1.1. Introduction.

Since 1945, agriculture has undergone a rapid intensification of production, to the extent that much developed world food has cost more fossil fuel energy to produce it, than there is food energy contained in it (Leach,1976; Pimentel et al.,1981). This poor energy ratio has sparked off an interest in renewable energy sources which may be employed on the farm itself, such as wind, solar, and (of particular interest to agriculture) biomass (White,1977).

One of the obvious biomass sources is animal slurry from housed animal units (Larkin et al,1981). It may be subjected to an energy conversion technique close to where the energy would be required. Of the techniques available, a good candidate for use with this specific material is anaerobic digestion, producing biogas, a mixture of methane and carbon dioxide which may be used directly for heat or light, or burnt in an internal combustion engine for the generation of electricity. Anaerobic digestion also has the benefit of conserving all the nutrients in the slurry, unlike any other treatment process.

The energy crisis in the early 1970's marked the beginning of a rise in interest in anaerobic digestion in agriculture in the developed world. The impetus for this was straightforward: any mechanism which could reduce energy imports at the farm gate, at a time when grid energy prices were unstable, was desirable. The extraction of biogas from animal wastes for use as a fuel, or as a means of electricity generation via an internal combustion engine, by the relatively simple expedient of subjecting the material to anaerobic conditions is appealing.

Ten years after this energy crisis, however, the results of the British Anaerobic Biomass Association's survey for the EEC indicate that there are

only 10 digesters operational on UK farms. (BABA,1981) This level of acceptability of digesters on British farms is not dissimilar to the proportions found in most developed countries. The exceptions are France, Germany and the USA where there are larger numbers (Stafford, Hawkes and Horton, 1980). These anomolies are probably due to variations in accessability of outside energy sources, and in the case of America to economies of scale due to high concentrations of animal wastes in feedlot meat production centres (Oppenlander, Cassell and Downer, 1975).When these low levels of adoption of the technique are compared to its importance in the developing world (Porter,1976) it is apparent that a motivation for installation exists in 3rd world agricultural systems generally not present in the developed world.

There are many reasons for this difference. The shaping and implementation of agricultural policy, and its relation to the social structure of food production in developing countries can play an important role. Sheshadri (1980) for example, emphasises the importance of social relations when dealing with the resource allocation problem of the utilisation of biomass for energy at a village scale in India. One of the interesting aspects of the FAO's visit to China (1977) to study organic waste recycling, is the reliance on community commitment to operate the system.

Also of importance is the low installation and running costs of these relatively simple digester designs due to subsidies, use of local materials and in many cases high ambient temperatures. Perhaps the most important reason for the widespread adoption of digestion in the developing world is the lack of access to any grid energy system.

1.2. Developed World Digestion.

It could be argued that developed world anaerobic digester technology and practice is overburdened with its own grandeur. While there is some attempt to move away from oversophistication in digester design (Jewell et al.,1978), it is apparent that a large component of the costs of digestion are in the fermentation vessel itself. Digesters which are designed to

raise the gas yield per unit of slurry add appreciably to the expense of the system, such that the marginal benefits far outweigh the increased costs.

The economics of developed world farm scale digestion indicate that the financial benefit is marginal. In the UK the digester itself may be eligible for a MAFF grant as it is classified as a measure for pollution control, subject to conditions. Notwithstanding this, the costs of installation and maintenance are high. Stafford and Etheridge (1981) indicate that the capital cost of the system must be reduced, or a real increase in the price of energy bought into the farm experienced before such systems become economic. James and Campbell (1983) in a comparative study of the economic benefits of a set of investment opportunities, show that the installation of an anaerobic digester gives a low performance compared to the purchase of either new buildings or additional livestock. Furthermore, Picken and Soliman (1981) suggest that an important recurring cost will be engine replacement if electricity generation is opted for. Engines receive no MAFF grants.

It is interesting to note that apologists of anaerobic digestion frequently make only cursory or no mention of digester economics. Meynall (1982) for example, in a text on planning a digester does not consider the problem of costs at all. Some workers indicate that farm digestion is only economic if the value of the digested slurry is allowed for. (Hawkes, Horton and Stafford (1976) have gone as far as to assume that undigested slurry has no value at all). It is argued that digestion improves the fertilising qualities of the slurry in conventional land spreading operations (Palz and Chartier (1980); Klass (1980), among others). The in vitro evidence (**Appendix** I), and related experience suggest that this may be the case.

1.3. Conventional Fertilising with Slurry.

The chemical characteristics of digested animal slurry suggest that it has greater nutrient, notably nitrogen, availability for plant growth compared to the equivalent undigested slurry.If this is the case, digestion can increase the value of the slurry by facilitating greater first season nutrient uptake, and increasing the precision with which manure applications are made, due to a more predictable response. A major drawback in using animal manures as fertilisers is the error in estimating the first season value (Cooke, 1975).

The received view on digested slurry application is that first season nitrogen availability is enhanced. This is the case for digested over undigested sewage sludge, where a 50% increase may be anticipated (ADAS, 1978). There is, however, relatively little quantitative information available concerning the value of digested animal wastes. Tietjen (1966) observed that the reduction in the C:N ratio by digestion improves the fertilising qualities. Some workers, on applying digested slurry have indicated a high first season availability in their results (Hutchinson (1972); Chow (1977)), but they offer no comparison with an undigested control applied at the same rate. Kuzelewski and Pentkowski (1962) in pot trials on oats and potatoes discovered no difference in yield between aerobically and anaerobically treated cow slurries.

Higher availability of nitrogen is, however, suggested by the increased proportions of ammonium nitrogen (Summers and Bousfield,1980) and protein nitrogen (Bellamy and Hughes, 1980), after digestion. These forms of nitrogen are relatively accessible to plants over the growing season, and increase during digestion at the expense of nitrogen associated with remaining plant materials (Badger, Bogue and Stewart, 1979). Non-protein, organic nitrogen in the organic amendments is less readily transformed to N forms available for plant uptake (Power and Legg,1978).

These factors, coupled with the reduced C:N ratio due to losses of carbon as CO_2 and CH_4 in digestion, indicate increased N availability. Meynall (1982), and Stafford, Hawkes and Horton,(1980) support this view, although Robinson (1976) suggests that the difference in available N

between digested and undigested piggery wastes is small.

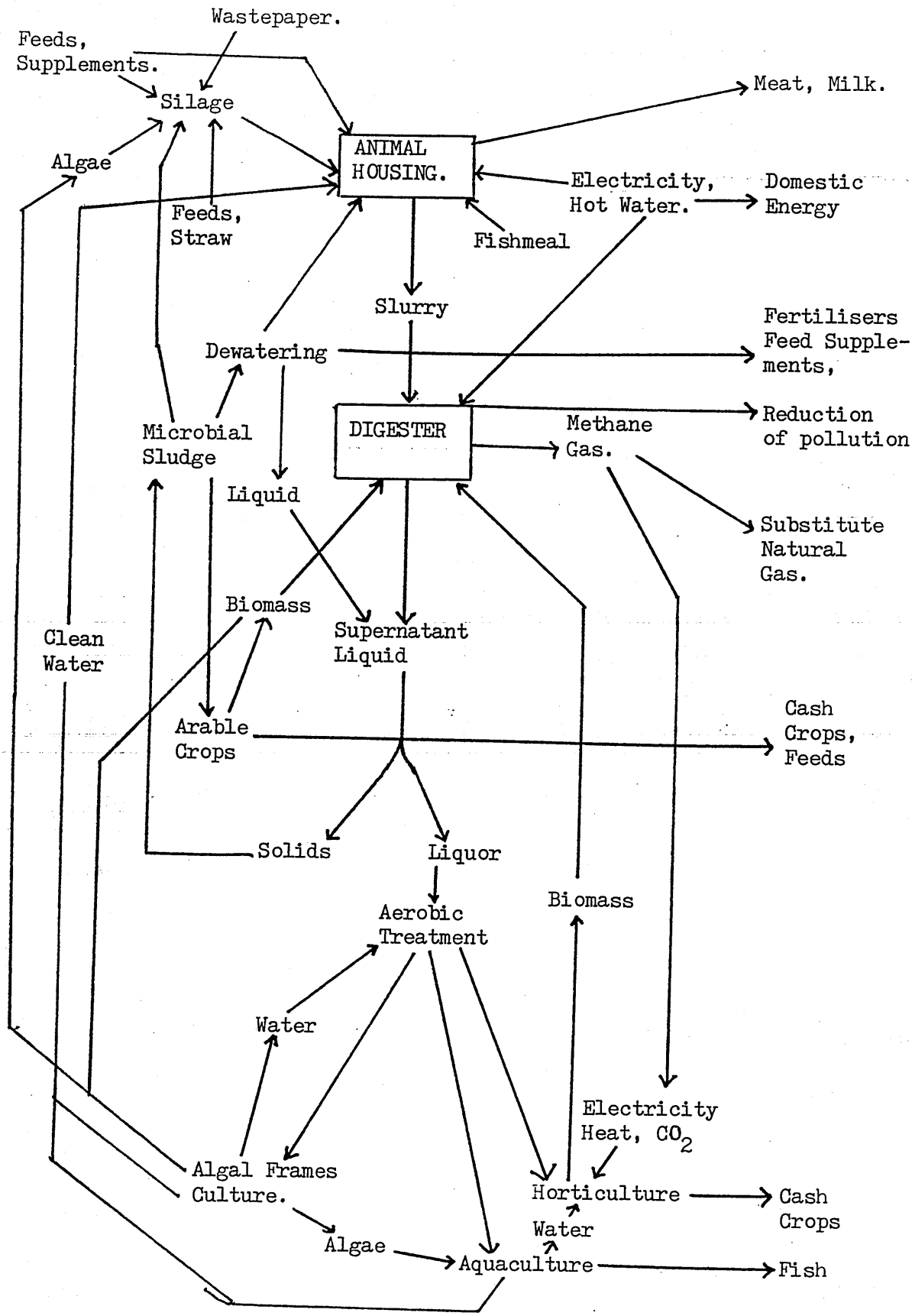
In order to establish whether a nitrogen availability advantage due to digestion exists, a pot experiment using ryegrass (Lolium perenne, S23), was carried out.
The experimental details and results are given in **Appendix II.** Whilst the experiment was not repeated, and was carried out under the somewhat artificial circumstances of a pot based trial, the results are unequivocal: no difference in first season nitrogen uptake between digested and undigested treatments is statistically detectable. The result is in accord with field scale trials over 2 years in the UK, employing digested cattle slurry on grass on the Wealden clay (S.Crocker, pers. comm.), and in Denmark on grain and root crops on a variety of soil types (Dam Kofoed and Klausen, 1983).

If digested slurry displayed enhanced availability due to digestion, then continued use as a land spread fertiliser would be desirable, as slurry spreading is an integral part of an animal based agricultural system. The downstream (non-gaseous) economic benefits of digested slurry would be felt without needing to employ additional capital. Such a slurry utilisation strategy would also make use of the easier slurry handling due to the reduced solids content (Morris et al.,1981). With no major benefits in nutrient availability, however, other options for using the slurry in order to give an economic benefit must be investigated.

1.4. Potential Uses of Slurry.

The idea of using ostensibly waste materials as the starting point for other food production purposes has been quite widely investigated. Church, Erickson and Widmer, (1973) have reported on the use of food processing wastes as a substrate for the production of fungal single cell protein. Similarly, Righelato, Imrie and Vlitos (1976) have proposed a low technology SCP producing plant employing agricultural wastes. Smith and Rothman (1981) indicate a potential for one of the most ubiquitous SCP sources- treated sewage sludge.

Diagram 1.4.a. Closed Cycle Farming (Bioplex) Walnut Tree Farm, Chediston, Suffolk. (after Bellamy).



More specific schemes involving the production of several products by using the solid and liquid components of the liquid for different purposes have been mooted. Forster and Jones (1976) suggested a 'Bioplex' model for the use of slurry for the production of food, with products ranging from hydroponically grown tomatoes, to mussels and single cell protein. Stead (1978) expanded this concept to municipal wastewater applications which allowed recycle of the nutrients not employed in the first food production option to facilitate complete utilisation.

Some efforts have been made to apply these ideas directly to the slurry produced by the anaerobic digestion of animal wastes. Marchaim et al. (1981) have reported on the Israeli attempts to use a farm based anaerobic digester as a means to produce not only energy, but animal and fish feeds, and a medium for the growth of horticultural produce. The most comprehensive appraisal of the potential of anaerobically digested slurry has come from Bellamy and Hughes (1980), who have described a system which is related to a particular commercial-cum-experimental farm. A diagrammatic representation of the proposals is given in Diagram 1.4.a.

One of the main problems with these systems, however, is that none are operational.While there have been quite a few models of 'Bioplex'-type systems published, very few of the suggestions have hard information on their technical or economic potential. The Office of Technology Assessment of the US Congress (1981) have pointed out with regard to anaerobic digestion:

> "More needs to be known about the difference between digested and undigested manure. The digested manure should be investigated in order to determine its value as a fertiliser, animal feed and nutrient source for aquatic plants. High value uses for the digester effluent, proved through thorough testing, could significantly improve the economics of anaerobic digestion"

It is apparent that the economic viability of developed world anaerobic digestion is under question. One of the ways to improve the overall status of the system is to utilise the digested slurry in such a way that the changes in slurry characteristics affected by digestion may be exploited, giving rise to a high value product.

2.1. Introduction.

Chapter 1 suggests that the economic viability of anaerobic digestion in the developed world is marginal. Furthermore, there are few proven benefits attributable to digestion in using digested slurry as a conventional fertiliser. Other ways of improving the economic performance of A.D. must be sought, therefore. It has already been indicated that the use of digested slurry in other food production enterprises effectively adds value to the slurry. It is appropriate to examine in some detail those enterprises which have been suggested in the literature.

In an animal based enterprise (such as would employ a digester) it is biologically desirable to shorten the food chain for the production of meat. The efficiency of protein production from meat is notoriously low (Spedding, Walsingham and Hoxey, 1981). A comparison of the annual protein production per unit of land area shows that crops are more efficient producers than any animals except rabbits. Furthermore, the indices of edible protein produced per unit of nitrogen input are very low for animals (Holmes, 1977).

While there is some evidence for increasing vegetarianism in human food habits (Wardle, 1977), the maintenance of the position of meat in the diet (in the developed world, at least) indicates a need to improve the efficiency of its production, if it is not to become increasingly anachronistic in food production systems.

Appendix I demonstrates that there are three distinct fractions in digested animal slurries-settleable solids, suspended solids and the aqueous phase. Pragmatically, normal farm waste management practice generates two fractions- coarse, settleable solids, and a supernatant liquor. Both of these products have been suggested as being suitable for

the production of animal feed materials.

2.2. Animal Feed Materials.

There are a number of proteinaceous feed materials which can be generated from one or other of the fractions of the slurry, using it as a nutrient and/or energy source. These are microalgae, produced either in a high rate algal pond, or in lagoons, obviously making use of the liquor phase; yeasts, utilising the same material, and worms, which make use of the coarse solid fraction.

2.2.1. Microalgae.

Microalgae have a very high level of productivity. Priestley (1976) estimates that the annual protein yield per hectare on a dry weight basis for Chlorella pyrenoidosa is 15,700kg, and for Spirulina platensis 24,300kg. Compared to conventional systems (1,680kg for clover leaf, 670kg for grass, and 100kg for milk), these two relatively extensively cultivated microalgae species demonstrate a very high protein production potential. The true protein content of these microalgae is approximately 60%.

While these values presuppose sophisticated control equipment, purpose built shallow lagoons and CO_2 enrichment, the impact of microalgae on the biological efficiency of meat production could theoretically be very large. Although the conventionally adopted substitution limit in the diet of monogastric animals is 20%, and pretreatment is neccessary to break down the sporopollenin cell wall (Garrett, Strain and Allen, 1976), this non conventional protein source is potentially of high economic value.

The concept and practice of growing algae on wastewater was developed some time ago, initially as a means of nutrient stripping. The first study was concerned with municipal wastewaters (Burlew,1953), and subsequent development for use with animal effluents has presented few problems. The main additional requirement is for rigorous mixing to prevent the settlement of the larger solids which could give rise to an anaerobic bottom layer, a potential source of instability in the biological system,

and a physical constraint to efficient operation by clogging (Dodd,1979).

As microalgae have a very high level of productivity even under uncontrolled conditions (a wastewater lagoon will self seed and bloom rapidly without the aid of environmental controls), their adaption to food and energy production uses has been relatively simple. Umstadter (1980) reports the harvest of Tilapia spp. from algae filled aerobic livestock wastewater lagoons which were initially stocked to reduce the self seeded microalgae which were causing a "choking" problem.

For similar reasons the variety of operations which microalgae can be involved in is large. The algae can be used as a feed for herbivorous fish in the pond itself. Edwards (1980a) suggests the use of Tilapia nilotica which will feed equally well on phytoplankton as on pelleted feed, and can tolerate the low dissolved oxygen concentrations which occur in the early morning in algal ponds. This use of fish as a biological harvesting medium reduces inefficiencies and added costs associated with mechanical harvesting techniques, and is most appropriate where capital (or the desire to employ capital) is a limiting factor. Simpson and Morales (1980) have reported similar work using a polyculture of fish in order to maximise protein removal from the pond. They also suggest the use of pond water as a source of irrigation water, so that residual nutrients may be utilised.

Alternatively, microalgae may be harvested from ponds or wastewater lagoons and used as a feedstuff. Chow (1977) fed a slurry of undried Chlorella produced in swine effluent lagoons to swine, and found no significant difference in gain and feed efficiency compared to a soybean meal and cottonseed meal control. While the feeding of wet material appears to work, health problems may arise if precautions in preparations are not taken, due to pathogen transfer. This is especially true of monogastric animals. Most workers propose harvesting and drying, notwithstanding the increase in costs. Anaerobic digestion can significantly reduce pathogen levels in animal slurries, especially thermophilic digestion (Stafford, Hawkes and Horton, 1980), and would be beneficial in this context.

Microalgae are well established as a feedstuff: Priestley (op.cit.) reports extensively on successful trials for ruminants, monogastric animals, and even humans, although Garrett, Strain, and Allen (op.cit.) point out that high lysine, and low methionine and cystine levels may cause feed formulation problems for monogastrics. Direct harvesting of microalgae is normally practiced when fish are being cultured, but preharvested algae may be incorporated into formulated fish feed. Shelef et al. (1976) achieved a 30% isonitrogenous substitution for fish meal in the diets of Cyprinus carpio and Tilapia galilea without loss in fish weight gain or health.

There appear to be benefits in growing microalgae on digested animal wastes compared to inorganic formulations. Short chain volatile fatty acids present as intermediate breakdown products from digestion may be used by the algae as a carbon source, although they normally fix carbon by photosynthesis. Chow (op.cit.), Venkataraman (1979) and Chung et al. (1978) all obtained higher yields of algae grown on digested effluent compared to inorganic controls of equal nutrient status. Chung and his coworkers suggested that bacterial decomposition of organic matter releasing CO_2 may have also been a factor in elevating yields. Oswald and Benemann (1980) corroborate this, indicating that this relationship is an important factor in pond productivity.

The logistical problems of microalgae production have largely been solved, due to the long history of application for food production. Harvesting problems can be resolved at a range of technology levels. Venkataraman (op.cit.) reports on an intermediate technology involving a hand operated pump passing the liquid through cloth filters. Elegantly, this system works due to species selection. The pond is seeded with Spirulina platensis, which dominates other, self introduced species. S. platensis is a long (500um) spiral which is easily captured by screeening, compared to Chlorella ssp. which are typically 8-10um long, and require more sophisticated techniques. Oswald and Beneman (op.cit.) suggest that for efficient harvesting dissolved air flotation, centrifugation, or chemical coagulation are required, while Dodd (1979) proposes a paper coated belt filter for the extraction of Chlorella.

A spectrum of technology options is also displayed in the provision of optimal environmental conditions for growth. While aerobic lagoons are successfully employed in operational systems, higher efficiency levels may be obtained with purpose built, high rate algae ponds, requiring mechanical mixing and CO_2 supplementation. CO_2 enrichment especially gives very high productivity benefits (Goldman, Dennett and Riley,1981), although the energy cost is high. For mixing, Oswald and Benemann (op. cit.) estimate 15Kw/ha/hr.

Given the capability for providing an optimal cultural environment, the first limiting factor is likely to be light. Garrett and Fallowfield (1980) suggest that for Northern Europe this will not preclude economic microalgal production. There is, however, a considerable yield reduction compared to more favourable climates. On the basis of their offered values, a 10cm deep pond and 12 hours of light gives a daily production value of 7.8g/M^2 of surface area. Oswald (1980) gives a value of 28g/M^2/day for a clear day in Manila, and Venkataraman (op. cit.) 15-20g/M^2/day for southern India. Microalgal production is obviously favoured by conditions of high light intensities and extended sunlight periods. The economic efficiency of Northern European production must suffer, all else being equal, due to light availability. Less capital intensive systems, in conjunction with aquaculture may be more appropriate for such areas.

Attempts at recycling algal biomass as a feedstock for further digestion have been made. Oswald and Beneman (1980) suggest that this would theoretically be economical, although Beneman and his coworkers (1978) failed to achieve comparable algal yields with recycled, digested algal effluent. The relatively low C:N ratio of the algal product suggests that microalgae are not particularly good producers of carbon as a digestion feedstock.

In terms of short circuiting the routes to protein production, however, microalgal production is one of the most attractive options available for digester residue utilisation. Subject to adequate controls being implemented for the control of predators on the microalgal population, the off-the-shelf technologies available indicate that from a biological and technical standpoint, this production system could slot into existing

agricultural practice with the minimum of disruption. The corollary is that, for intensive production the land, capital and labour requirements are large. In an attempt to evaluate the potential of controlled environment agriculture, de Bivort, Taylor and Fontes (1978) indicate that only high value vegetable crops can sustain the costs incurred in such a system.

2.2.2. Yeast.

Yeasts have been shown to be able to grow on the supernatant of anaerobically digested animal wastes, using the ammonium nitrogen, and the short chain (C2 to C6) volatile fatty acids which are present as intermediate degradation products (Henry et al.,1976). Irgens and Clarke (1976) achieved yields of 7.3g dry weight of Candida utilis grown on 1 litre of digested effluent diluted by a factor of ten, with 1.8% molasses as a supplementary carbohydrate source. Taya et al. (1979) obtained 7.7g of C.utilis from 1 litre of the supernatant of anaerobically digested cellulose wastes. Scaling up to process production of yeast has not, however, been achieved, and the separation of the product by centrifugation is expensive. Boersma et al. (1978) suggest that a self flocculating strain Candida acidothermophilum may alleviate that problem.

Yeast is an ideal component of animal feedstuffs, having a true protein content of approximately 45%, coupled with a high biological value. It is also very productive: Thaysen (1957) in a comparison of different protein production systems estimates that in 24 hours a bullock will produce 0.4kg of protein; and 500kg of soy plants produce 36.6kg. For the same weight of yeast over the same period, under 'ideal conditions', 50,000kg of protein would be produced.

The 'ideal conditions' are the rub, however. While yeast will grow well on digester supernatant and, unlike microalgae, do not require light, the provision of optimal growth conditions would prove expensive. The basic system requirements for yeast production are the provision of a controlled environment chamber, sterilisation of the feed liquid, and heat. The process controls would be more sophisticated than those required for anaerobic digestion. This technology level is probably inappropriate for

most developing world applications, and would require technical skills unfamiliar to most developed world farmers.

If anaerobic digestion is seen as an intermediate step in the raising of skill levels in agriculture, and the long term ambition is the development of farm scale biotechnology to the extent that full-time technical labour may justifiably be employed, then yeast would play a role in farm controlled animal feed production. For the present, the bioengineering requirements of the process need further investigation before implementation would be possible.

2.2.3. Vermiculture and Composting.

Although conventional fertilising of crops is not the most efficient use of nitrogen in digested slurry, there may still exist a need for the application of an organic material, for the maintenance of soil structure, the deterioration of which is one of the most important problems for the long term sustainability of many agricultural systems (USDA,1978; Greenland, 1970).

Where soil conditioning is an important component of agricultural strategy, and an attempt is being made to divert the effluent produced by anaerobic digestion from land application to another food production system, the need for a soil conditioner may be satisfied by separating the coarse solids in the effluent, and spreading those. The solids may either be applied directly, or composted. Direct application may cause germination inhibition due to the presence of phenolics and short chain fatty acids (Wang, Yang and Chuang, 1967), so an ageing or composting process is favoured. Composting oxidises the the fatty acids and transforms the nitrogen to the more acceptable nitrate form. Oxidation can lead to nitrogen losses of up to 25% (Poincelot, 1975), but if the solids are considered a carbon, rather than a nitrogen source, these losses are acceptable.

An alternative proposal for the production of a 'compost-like' material (Rijkens, 1980) is by a modified digestion process involving the leaching of the coarse solids of animal wastes, and passing the leachate through a fixed bed reactor. The proponent of this system expects that energy

production would be little affected, and a soil conditioner is produced.

A compost like residue is also generated if the solids (after removal from the slurry) were to be used as a feed material for earthworms (Eisenia foetida is most frequently grown by this method). The worms, when mature, are used as a protein source in animal feeds (having a crude protein content of 60% on a dry weight basis). Under controlled environment conditions production of 6685kg protein per hectare of worm bed may be achieved anually (Hartenstein, 1981). 75% of the nitrogen in the substrate is incorporated into the worms, (Spedding, Walsingham, and Hoxey,1981), leaving a worm worked material as a soil conditioner.The worms do not grow in anaerobic conditions, however, and ammonium nitrogen is toxic (C.Edwards, pers.comm.), so solids removed from digester effluents require an ageing process before use. Vermiculture is probably better suited to aerobically treated animal wastes.

2.2.4. Refeeding the residue.

The most direct means of shortening the food chain in the production of animal feeds is direct refeeding of the faecal materials, either cross- or same species. This may lead to problems of health, with reinfection of any disease factors which may be present. Anaerobic digestion may have benefit of sanitizing the slurry under certain conditions (Stafford, Hawkes and Horton, 1980). Some attempts at refeeding fractions of anaerobically digested animal slurries have been made, but with mixed results.

The concept of refeeding is not new; and considerable work has gone into defining the nutritional value of animal faeces, together with the health criteria and processing methods for it (Bhattacharya and Taylor, 1975). While this experience is important when considering the possibility of using animal based digester residues for refeeding, the application of this material has generated a discrete set of problems.

At first sight, digested animal slurry should offer benefits in animal feeding not available with undigested materials. The increased proportions of nitrogen as ammonium nitrogen, and protein nitrogen due to digestion (as indicated in Appendix I) should enhance the feed value of the material, in ruminant nutrition at least. In practice problems associated

with processing, and and the constraints applied by the physico-chemical form of the material, give rise to serious difficulties. A discussion of the (albeit scarce) literature on the subject highlights some of the problems.

Two categories of feeding are apparent in the literature: the use of separated,dried solids, and the incorporation of wet material into preformulated feed. Lizdas,Coe and Turk (1981) used the solid fraction of digested cattle slurry obtained with a solid bowl, decanter type centrifuge as a substitute for cottonseed meal protein in the diet of (only) 2 Charolais crossbred steers. The material contained 22% dry matter, with 25% crude protein, and provided 21.7% of the dry weight of the diet. The residue gave a protein digestibility coeffficient of 72.7%, compared to 81.0% for cottonseed meal, an effective coefficient of 90% of that of the meal. In a second trial, caked, digested slurry solids were fed to 21 cattle over 79 days, at a rate of 4.06% of the diet on a dry weight basis. A quoted daily weight gain of 3.3 lb, with a feed conversion ratio of 6.9 was shown. Control values were not offered, and the digested material was supplemental to the normal diet.

Hashimoto, Prior and Chen (1978), used dried centrifuge cake from digested cattle slurry, as a substitute for alfalfa hay up to 20% dry weight of the diet fed to sheep. With increasing substitution N uptake was reduced. Faecal N as a percentage of N intake increased from 36.6% in the control, to 45.5% at the 20% substitution rate. A regression analysis indicated that faecal N increased by between 0.3 and 0.4 percent for each 1 percent increase in centrifuge cake, indicating that a component of the N in the cake was less digestible than N in the alfalfa hay. In a companion experiment (Prior and Hashimoto,1981), centrifuge cake was substituted up to 20% by weight in a cattle diet, substituting for all other dietary components. Faecal N as a percent of N intake increased from 41.5% in the control, to 48.6% at the 20% level, and ash digestibility fell from 51-60% in the control diet, to 28% in the 20% diet, a result mirrored by the sheep work. Regression analysis suggested that only 11.3% of the ash in the centrifuge cake was utilisable.

This work demonstrates the difficulty associated with solids collection. Centrifuging will allow the capture of up to 60% of total solids in the slurry, which is used as roughage in the ruminant diet, with some ammonium nitrogen and soluble protein in the liquid remaining in the cake. Nitrogen associated with the coarse solids will be relatively unavailable for use by the ruminant. The high correlation (r=0.96 to 0.99) between crude protein content, and digestible protein content established by Smith (1973) for undigested animal wastes does not appear to hold for the cake fraction of separated, digested waste.

There is, however, some evidence which contrasts with this trend. Saxena and Ranjhan (1983) in a medium-term feeding trial on sheep, using the dried, complete slurry found no difference in dry matter and crude protein digestibilities on a regime of a 30% isonitrogenous substitution for wheat bran.

The nitrogen present in the coarse solids is relatively unamenable to microbial attack, having previously passed through a ruminant and an anaerobic digester. This also means that, while the gross energy of the coarse fraction is quite high, the metabolisable energy value is low. The cellulose/hemicellulose fraction has been degraded, leaving the lignin and lignin derivatives. In addition, the high density ash fraction is captured and, as demonstrated above, is rejected by the animal. Centrifuging, and other 'off-the-shelf' separation technologies, effectively collect that fraction of the slurry which is low in utilisable nitrogen and metabolisable energy, and is best suited for a different use, such as landspreading or vermiculture.

This problem is avoided by feeding either the reject liquid from the separation, or the digested slurry in its entirety. Marchaim and Criden (1981) report successfully feeding thermophillically digested cow manure (at an unspecified percentage in the diet) to 3 sheep for 9 months, with no toxic effects. The slurry was mixed wet with other dietary components, after the (unsurprising) refusal of the sheep to drink it. In a following experiment lasting 18 weeks, "normal performances" were found with 4 Holstein heifers with 25-30% dry weight substitution in the diet. While no information on the preparation is offered, the description suggests that

the bacterial rich, liquor fraction was employed.

Lizdas, Coe and Turk (1981) in a trial involving 20 cattle over 69 days, used the liquid fraction of digested cattle slurry at 2.33% (dry weight basis) of the diet. They showed a daily weight gain per animal of 3.7lb, and a feed consumed to weight gained ratio of 6.3:1. Unfortunately, no data for a control population are offered, and the digested material was supplemental to the normal diet.

In the best experiment of its type, Prior and Hashimoto (1981) ensiled the complete wet effluent with sodium bentonite (to increase feed efficiency) in the ration at a 6.5% dry weight basis substitution, for sheep. Significant reductions in ash digestibility, apparent N digestibility, and dry matter, organic matter, and gross energy uptake were observed. (They did not, however, discover any quality differences in the carcasses on different diets).

By feeding the whole slurry including the crude solid, ash-rich fraction, problems in nutrition are still encountered. Furthermore, feeding of the wet material has problems of its own. Prior and Hashimoto (1981) note that a wet ration will mould very rapidly in summer, freeze in winter, and have a much shorter storage life. Richter (1979) discovered a significant reduction in dry matter intake in steers fed wet cake, substituting 30% on a dry matter basis for the normal diet, and Spedding, Walsingham and Hoxey (1981) point to increased risk of disease, and problems of palatability, handling and distribution when wet faeces are employed.

No attempts have been made to feed the protein rich liquor to monogastric animals. Whilst wet feeding of pigs is relatively common, the dilute nature of the residue, and the presence of ammonium nitrogen in solution which is of no use to a non ruminant, probably preclude its use.

In economic terms, the high value of the protein rich product make it worth pursuing, notwithstanding technical difficulties in its removal. Marchaim and Criden (1981) estimate that when 15-25% of a ruminant's diet is replaced with digested cattle slurry, the value of the slurry is 50-75% greater than its value as a manure. Lizdas, Coe and Turk (op.cit.) consider

that the economic worth of digester solids is between 50 and 60 percent that of cottonseed meal, although they do not specify whether upstream preparation costs are offered to compare the true price. Hashimoto, Prior and Chen (1978) in a paper estimating the economics of anaerobic digestion, assess the net energy value of the gas produced by the fermentation of cattle manure to be worth 7.1 dollars per head per year, assuming the natural gas price to be 1.85 dollars per gigajoule. In comparison, the protein value of the effluent, based on essential amino acid content, is 82.9 dollars per head per year. Even if this estimate is overgenerous, the importance of the residue as a feedstuff/concentrate outweighs that of the gas.

The problem of extraction and application still remains, however. It appears that unless extraction of the protein from digested animal wastes is approached with the specific need of producing an animal feed material, the potential of this economically and energetically valuable material may not be realised. Prior and Hashimoto (op.cit.) consider that under present circumstances only 5-10% substitution may be achieved if near maximal animal performance is to be maintained, a point borne out by other workers (Bellamy,unpub. data). More problematic however, is the long term impact on digester performance. Hashimoto, Chen and Prior (1978) point out that biodegradability of a digester feedstock is dependent on the animals' diet. Increasing roughage decreases biodegradability substantially, and will reduce gas yields. Long term refeeding of the coarse fraction (which will pass continuously through animal and digester) will bring about a systematic reduction in gas yield. Aspects of these problems are best summed up with a general comment from Hashimoto, Prior and Chen (op. cit.):

> "The other area requiring technology development is more effective systems to recover the valuable protein. We have found that only about 20% of the protein can be recovered by centrifugation. Incorporating the fermentor effluent directly with the grain and additives is an effective way to utilise the protein; however, the high moisture required for complete replacement of supplemental protein (e.g. soybean meal) may cause handling problems like freezing in the winter, and short bunker life in the summer. Also, refeeding both the fermentor effluent and the centrifuge cake causes statistically significant decreases in ash digestibilities. Thus, systems which would separate the high quality protein from the inorganic salts and water would be beneficial."

While there are problems related to its implementation, the benefits from this option (i.e. direct use of the protein as a feedstuff),are potentially very large, particularly if a separation stage is employed allowing use of the other fractions, thereby diversifying the food production activities, and increasing overall nutrient use efficiency.

2.3. Diversification.

In addition to those options which are geared to the production of feeds which are directly related to the main enterprise, there are others which are quite dissimilar, and yet may also be used in conjunction with digested slurry.

2.3.1. Fishculture.

There are two generic types of fishculture, both of which may use digester residues as an input: open water systems which are fertilised with organic wastes, with or without supplementary feed material, and intensive cultivation systems relying on formulated feeds (which consequently attain better feed conversion ratios). While intensive systems are more productive both in terms of area and feed supplied they are more costly, and the bulk of freshwater fish produced worldwide is raised in openwater systems.

Open water aquaculture employing organic wastes has been practiced for millenia (originating in China), and very high yields may be obtained under certain conditions. In the Peoples' Republic, for example, yields from this cultural method far exceed those achieved in other parts of the world (Tapiador et al.,1977). Considerable effort is being made to increase the efficiency of such systems, but the main reason for restricted yields is the lack of understanding of the food web and nutrient relations of a fertilised pond. Wohlfarth and Shroeder (1979) highlight the problem:

> "Pond management systems have been arrived at empirically but our understanding of the dynamics of the natural food web is deficient.... A knowledge of the various pathways of the natural food web might enable stocking and harvesting the different polycultured species to be done in a more rational manner and result in increased fish yields, decreased hazards of fish killed due to anoxia and more efficient utilisation of the matter contained in the manure."

The polyculture strategy (stocking a pond with several fish species) attempts to increase efficiency of manure usage, as different fractions are available to different fish, depending on feeding habit. Schroeder (1979) reports that in a manured polyculture system with Tilapia aurea, silver carp, and common carp, the first two grew better than the common carp. Silver carp is a filter feeder, Tilapia a detritivore, and common carp is a pelagic feeder. The relatively high feed conversion ratio obtained with Tilapia and silver carp in this example suggest that the food chain between manure and fish is short, and that direct consumption of micro-organisms is taking place. A waste with a high proportion of organic fines would favour these two species.

Attempts to match species for polyculture with the organic wastes used for fertilising ponds have been made. Wohlfarth (1978) reports on trials which vary the organic additions made to polycultures containing different fish. Christiensen (1978) has attempted to match waste materials with the best species to utilise them by feeding habit and waste characteristic. This latter work assumes direct consumption and makes no attempt to consider the effects of the dynamics of the (admittedly very complex) pond system. In practice a diversity of food options are created for pond fish:-direct consumption of the coarser solids of the organic waste (generally manure), together with some exploitation of the microbial protein source present in the waste. Decomposition of added solids gives rise to a native microbial population which may be consumed by bottom feeders, and free mineral nutrients from the manure, or released from decomposed solids, are utilised by phytoplankton. These microalgae are fed on directly by fish (Edwards,1980a), or grazed by rotifers and ostracods, which are in turn consumed. The relative proportions of these products, which is dictated by the nature of the wastes used, determines the optimal polycultural mix (Hepher and Pruginin,1981).

This is rather a simplistic model of pond dynamics. Feeding habits change with population age for example (Hepher,1979). However, if it is accepted then generalisations on the use of digested, rather than undigested animal slurries in ponds may be made. The loss of approximately 50% of the volatile solids alters the carbon balance: less nutrients are associated with plant derived organic matter, and are consequently more

rapidly taken up by phytoplankton. The phytoplankton may use as a carbon source the short chain fatty acids which are present as breakdown products in digestion. The coarse solids will tend to be less readily decomposed, and have a lower metabolisable energy content than undigested solids due to the increased proportion of lignocellulosic material. The high level of single cell protein can be exploited by bottom and filter feeders. The lowered carbon levels will give a much more rapid response in primary production to the input compared to undigested wastes, as is the case with inorganic additions (Wahby,1974). This indicates that lower levels of digested, as opposed to undigested, slurries should be applied to the pond, on an N for N basis.

These conclusions are somewhat conjectural, and of a low level of resolution. This is not unusual, however, with the present level of information on pond loading. Edwards (1980b) points out that many generic waste types have been used for pond fertilisation, but data collection is frequently patchy, and then confined to final yields. Such a crude assessment of the effects of different waste types preclude efficiency improvements, or understanding the impact of these variations. Animal manures altered by anaerobic digestion are merely alternatives in this suite of potentially useful materials. If the guidelines for pond loading rates are adhered to, then such materials may be used, notwithstanding the low level of understanding of the mechanisms involved.

In contrast to the empirical and occasionally haphazard open water fish culture systems, intensive aquacultural practice has, through the imposition of optimal, or near optimal environmental conditions, achieved high growth rates. This is partly possible by precise feed formulation using high protein inputs such as fish meal, and meat and bone meal. Provided the essential amino acid, crude protein and metabolisable energy requirements of the fish are met, most proteinaceous materials may be used in the diet. This includes single cell proteins, as nucleic acid content is not a constraint in fish nutrition.

The high cost of dietary components has led to the search for suitable alternatives, (as a replacement for fish meal) (Hepher, Chervinsky and Tagari,1971). Bergstrom (1979) substituted Pruteen (methanol fermenting

single cell protein), and Pekilo protein (a fungal protein source grown on paper wastes) at 33% and 50% of the diets of Atlantic Salmon without adversely affecting fish growth, but found depressed growth and increased mortality when using Candida utilis yeast as 50% of the protein source. Beck et al.(1978) found no ill effect in the performance of trout using alkane grown yeast and methanol fermenting bacteria, at an isonitrogenous substitution level of 30%, with similar results for yeast, amino acid and chicken by-product at a substitution level of 60%. For herbivores, Atack, Jauncey and Matty (1979) achieved a 30% substitution for conventional protein sources with bacteria, yeast and algal cell protein with some growth and protein utilisation benefits over fish meal and soybean meal.

Some effort has been made to use 'waste' materials as protein substitutes. Kerns and Roelofs (1977) using poultry manure as a 12.5%, 20% and 27.2% substitute for carp, found better growth with a control diet of wheat, fish and soya meal. Similarly, Lu and Kevern (1975) substituting poultry manure for trout feed in the diet of channel catfish showed a better response with the control diet than with 30%, 70%, and total substitution. For cattle manure, Shiloh and Viola (1973) found reduced growth rates with 10% and 20% substitution in a diet of fish meal and soya meal. They concluded that fibre may not be used for energy by carp, and, moreover, is a burden on the digestive system, requiring energy for expulsion whilst occupying space.

As Shiloh and Viola's work indicates, cattle slurries are not suitable protein substitutes as a high proportion of nitrogen is unavailable, being combined with plant materials. The lack of success achieved with poultry manure may be attributed to the high levels of mineral nitrogen, notably ammonium-N. Direct formulation with these materials offers no opportunity from transformation of nitrogen which is unavailable for the fish, to useful forms, as occurs in pond culture. Formulated and pelleted feeds need to be readily utilisable. Microbiologically processed wastes, with high levels of single cell protein as a by product meet this requirement. Tacon and Ferns (1976) used activated sludge single cell protein (ASCP) at 5%, 10% and 20% substitution on a weight basis for fish meal and wheat middlings for trout, and achieved a higher growth rate with 5%, and comparable rates with 10% and 20%, compared to a control diet. Tacon (1978)

achieved a 33% substitution on an isocalorific and isonitrogenous basis for soybean meal and wheat middlings for trout without reduction in growth rate. For common carp, Anwar et al. (1982) estimate that a 40% substitution of ASCP for a bran-cottonseed mixture may be achieved without growth rate reduction.

The success of feeding fish on cattle manures depends on the fish chosen. Cellulose users such as Tilapia spp. and grass carp would be able to use the coarse solids as an energy source. For such fish, digestion would be disadvantageous as any residual cellulose is removed. The coarse solids of the slurry, whether they are digested or undigested, are of no value to such fish as common carp. These factors, coupled with the high ash content, preclude the coarse solids from being used as an alternative protein or energy source in formulated diets. The suspended solids, containing most of the organic nitrogen, and a high proportion of the microbial protein, being generically similar to ASCP, may be of value as a substitute protein source.

2.3.2. Hydroponics.

Conventionally, hydroponics operates within a clearly defined set of cultural conditions, especially liquid flow rates, nutrient levels in the liquid, and the ratios between those nutrients. Deviations from these norms, however, do not preclude the use of this technique, and effluent waters may be used to produce horticultural and market garden crops.

Early work with hydroponics using non-conventional liquids concentrated on the stripping of nutrients from secondarily treated wastewaters prior to rerelease. As the reaching of water release consent conditions was more important than the production of plants, grasses were used (Law, 1969). More recently, however, the same liquid has been used to generate economically valuable products. Sias and Nevin (1973) successfully grew leisure plants, and Wallace et al. (1978) have produced tomatoes. A more ambitious project involving complete liquid clarification (including the removal of viruses) with reverse osmosis, has employed sugar beet in order to come to terms with high sodium levels (Winfield,1981). The effluent from intensive fish culture has also

successfully been applied to tomato production (Lewis and Yopp,1977).

Effluents like municipal wastewaters have, however, relatively low nutrient and conductivity levels compared to the optima recommended for hydroponics use. Schwartz (1973) recommends the adddition of nutrients to prevent deficiencies occurring, but hydroponic plant production can (demonstrably) operate at these low nutrient levels. This is possible if an open ended system is employed to prevent the rapid nutrient depletion that would occur if a recirculating system were used. An open ended system entails running the liquid down gently inclined hydroponics beds or channels until the nutrient content is depleted. This condition will be demonstrated by increasingly severe deficiency symptoms appearing in the plants.

With such a high throughflow (Wallace, Soupi and El Gazzar (1979) used a system with a throughput of 27,000 litres per day in a single bed), reduction of the typically high pH of the wastewater is not economically feasible. Micronutrient deficiencies related to pH are rectified either by foliar feeding (Patel and Wallace,1976), or by the introduction of the deficient nutrients, notably iron, into the rooting medium (Winfield and Bone, 1981).

Digested animal slurries which have had no secondary treatment differ significantly from municipal wastewaters, however. High electrical conductivity and nutrient levels preclude its direct use in hydroponics due to osmotic pressure problems and toxic nutrient concentrations. Sludge hydroponics (over a gravel bed) has been suggested by both Fry (1974) and Saithanathan (1977) as an appropriate technique for digested animal effluent, but no data are offered in either paper. The efficiency of such a system is likely to be low due to seepage and ammonia volatilisation losses, should the plants escape physiological and toxicity problems.

In order to make the digested effluent suitable for hydroponic use, the conductivity must be reduced from a level of between 6 and 8 mmhos cm^{-2} to between 2 and 3, and the pH from a level greater than 8, to between 6 and 6.5. Provided these conditions are maintained, and the effluent water has a "reasonable" balance of plant essential nutrients (Cooper,1979) then a

recirculating hydroponic system can be operated without nutritional problems. No advantage is to be gained by further dilution for open ended system usage, due to the needs of increased equipment and land, and problems specifically associated with that method.

As a food production system, hydroponics has much to commend it. Nutrient utilisation is very high. Garraway (unpub.data) in work on aerated pig slurry for tomatoes established an N usage of between 90 and 100%. If a horticultural crop were to be chosen (such as tomatoes) a high value, easily marketable crop is produced.

In order to establish whether digested pig slurry may be used as a hydroponics medium, the supernatant from sedimented slurry was used to grow tomatoes. The experimental description and results are offered in **Appendix III** . It is clear from these results that three major barriers exist to its implementation:- the difficulty in controlling the pH when the slurry has such a large buffering capacity; the presence of ammonium nitrogen, and phenoxy compounds and high BOD_5 inhibiting plant growth and yielding potential.

These difficulties suggest before use in this context, digested animal effluent would require a secondary (oxidation) treatment before it would be appropriate for use in this context.

2.3.3.Aquatic Macrophytes.

The proliferation of aquatic macrophytes which occurs in eutrophic waters has been exploited for the stripping of nutrients from municipal wastewaters, mainly in the U.S. The most commonly employed species are Eichhornia crassipes (water hyacinth), (Alsten,1980), and Spirodela polyrhiza (giant duckweed) (Sutton and Ornes,1977). They can only be used where sufficient areas of contiguous low lying land are available to accomodate the beds needed for sufficient plants to strip the nutrients from large, regularly occuring volumes of wastewater (Golueke and Diaz,1981). The product is composted and landspread, but the emphasis is on nitrogen and phosphate removal to achieve water release consent conditions (Cooley and Martin, 1978).

The plants may also be used as a feedstock for anaerobic digestion. Hanisak, Williams and Ryther (1980) achieved 0.4 litres of gas from 1 gm of water hyacinth, approximately 2/3 the gas expected from animal manures, and suggest that the high productivity of this crop makes it eligible as a biomass source.

In addition to energy and soil conditioning applications, aquatic macrophytes may be used as an animal feed. Water hyacinth has been used for this purpose in China (FAO,1977), despite the high roughage and low crude protein content (12%). Protein analysis of duckweed (Lemna minor) indicates its suitability as a feed component and it may, with some pretreatment be ensiled. Selection of appropriate species for this process is at a preliminary stage, however.

Aquatic macrophytes display a considerable aptitude for water clarification. Micheli (1979) using E.Crassipes demonstrated a COD reduction of 97% and ammonium nitrogen reduction of 98%, with influent and effluent levels of 1,700 and 740 respectively, in digested pig effluent. Similar, though less spectacular results were obtained with L.minor and Salvinia (no species given). No indication was given, however, as to the fate of nitrogen, some of which is likely to have been volatilised, and no control was offered to establish growth efficiency.

Aquatic macrophytes have a high productivity level: Musil (1977) suggests a maximum specific growth rate of 0.114g fresh mass per day at 25 C for both E.crassipes and Salvinia). Despite this, there are considerable barriers to implementation at farm level. An extensive area of flat land with a plentiful supply of water would be required to service even a small percentage of the output of a farm scale digester. Where a digester is used for winter housed animals, a time difference exists between effluent production and its use. For permanently housed animals storage capacity would be required for the winter period. This indicates that aquatics may best be used in tandem with other enterprises, acting as a final effluent polish following a first utilisation. Even under the most favourable of circumstances, however, purpose built cultivation beds, and costly harvesting facilities (Bassham,1977) must be provided for plants whose feed value is less than that of grass. On a farm scale, it is

unlikely that full utilisation of the equipment would be achieved.

2.4. Discussion.

2.4.1. Options.

A summary of the possible food production enterprises arising from digested slurry is given in diagram 2.4.a. The complete slurry may be used for extensive aquaculture, and extensive microalgal production by pond loading. It may also, obviously, be land spread in the conventional way. If one of the conventional techniques is employed a coarse solid, and a liquor are generated. The coarse solid has been incorporated into ruminants' diets, but with limited success, and is probably better suited to composting, for direct cycle on the farm. It has also been suggested that this material may be useful as a peat substitute in market gardening . Vermiculture is also being investigated.

The liquor has been used (in an experimental context) for the culture of yeast; as a medium for the hydroponic production of tomatoes, and a nutrient source for aquatic macrophytes. Its use is well established for the production of microalgae.

Table 2.4.b. gives some indication of other considerations which may have some impact on the selection of these options. The figures given are generalised, having been generated on a suite of assumptions on the composition of the slurry and reduction in solids content due to digestion. Some of the yields are extrapolated from bench scale experiments, while others are based on figures obtained from the warmer parts of the USA. There is also no allowance for benefits accruing by not having to spread the slurry. The table also shows the difficulty in establishing 'common ground' for a comparison of the options. The choice of a downstream food production enterprise may be made on the basis of a desire for high nitrogen utilisation, the quality (or quantity) of the protein product, the available area, low or high labour or capital input, or the value added to the product.

Diagram 2.4.a. The Range of Food Production Applications to which Anaerobically Digested Animal Slurry may be put.

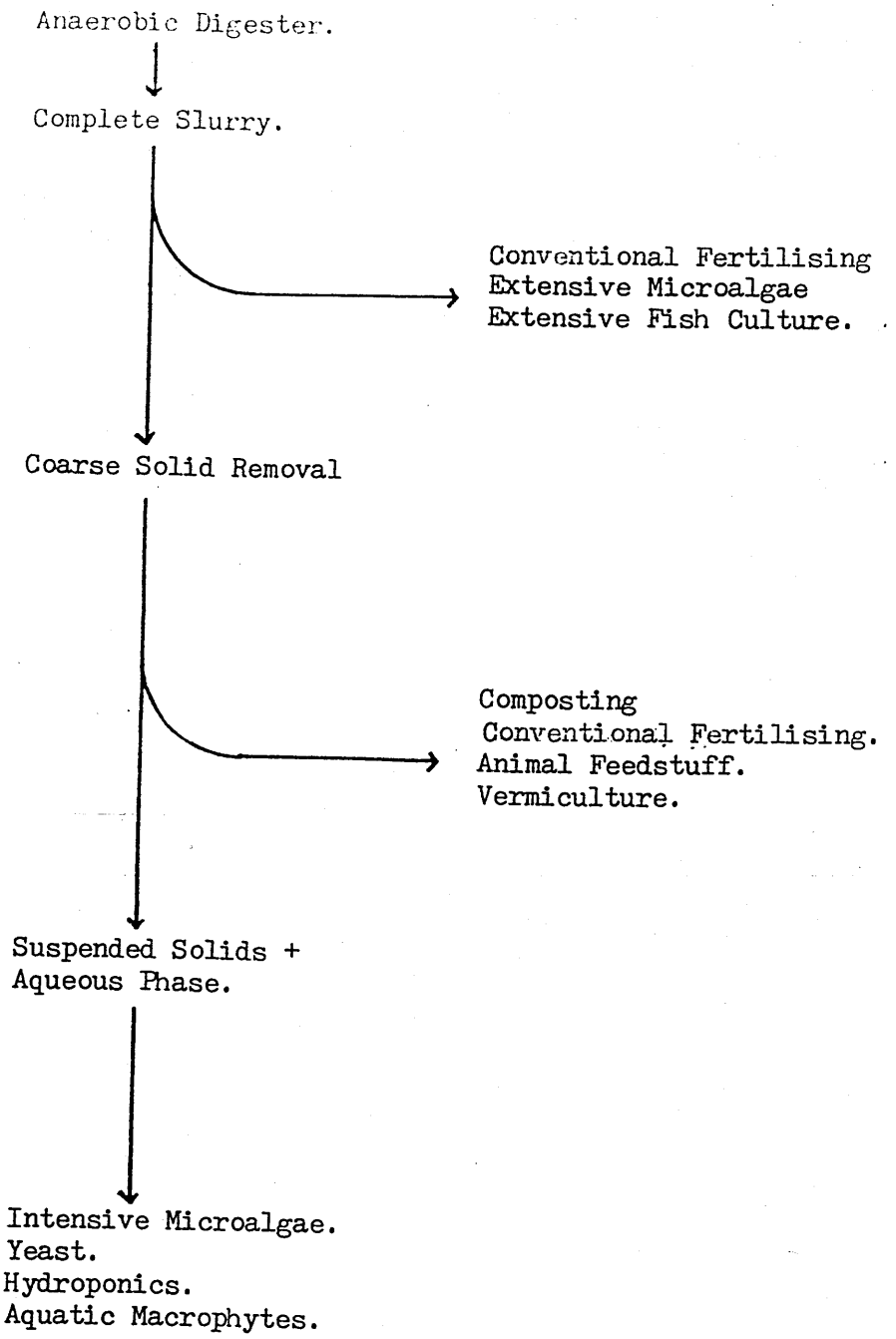


Table 2.4.b. Some Considerations on the Application of Digested Slurry to Food Production.

System.	Estimated Primary N Recovery (%).	Product.	% Protein.	Use.	Area required for a given output (a).	Quantity of product from (a) tonne/yr.	Value of product £/tonne.	Special Equipment	Labour Needs. (b).
Complete Slurry									
		Grain		(Human Food)		100-300			
Land Spreading.	50-55	Forage	15-30	Animal Feed.	50-125	1000-2000 (c)	50-150	None.	L:M
Extensive Aquaculture.	35-40	Fish, mainly Carp spp.	50	Human Food.	30-40	30-80	500-750	Ponds, harvesting facilities.	L:M
Extensive Microalgae	35-50	Algal slurry, or Air/Sun dried.	50-60	Animal Feed: Protein source.	5-15	120-170	100-200	Ponds, separating equipment (drying beds).	L:M
Sludge Hydroponics.	25-35	Forage	12-18	Animal Feed.	2	250-500	50-70	Pumping Equipment Gravel beds (Greenhouses)	M
Solids.								Separator.	
Composting.	50-85	Soil Conditioner.	-	Soil Conditioner.	¼	120-150	20-40	None.	L
Vermiculture	75	Soil Conditioner, Worms.	-, 60	Soil Conditioner, Animal Feed: Protein source.	¼	15-20	20-40 100-400	Worm Beds, (tunnel) Separator.	L:M
Ruminant Refeeding.	50	Roughage.	15-25	Animal feed: Energy source.	¼	130-170	50-70	None	L
Liquid.									
High Rate Microalgae	80-90	Algal cake.	65	Animal Feed: protein source.	1-2	150-200	100-200	Ponds, stirrers, Harvesting and Drying facilities.	M:H
Hydroponics.	80-100	Horticultural Produce (e.g. tomatoes)	-	Human Food.	20	2000-3000 (c)	300-500	Greenhouses, NFT equipment, gen. Hort. needs.	H
Yeast	95	Yeast Cake	50	Animal feed: Protein source.	¼	60-65	200-400	Fermentation vessel, separator and steriliser	M
Aquatic Macrophytes	50-90	Forage, Compost or A.D. Feed.	10-18	Animal feed. Soil Conditioner.	10	800-900	20-50	Large area low lying land, harvester.	M

(a) The slurry output from a 200 cow, or 1000 pig farm, in hectares.

(b) Labour requirements:
H=High
M=Medium
L=Low.

(c) Fresh weight basis.

Notwithstanding the broad spectrum estimates that have been made, the table reinforces the view that utilisation of the spent slurry may make a significant contribution to the economics of digestion. One limitation of the table is that no estimate of the capital cost of each option has been made, mainly because of the variety of accounting methods, and the variation in available capital for investment from year to year, which is such a feature of agricultural economics. Crude estimates of capital cost and amortisation may be made, but details cannot be supplied, as the lack of technical information forestalls such analysis at present.

What also emerges from this table is the observation that it is unlikely that all the output from a farm of 200 cows or 1000 pigs could be used entirely for one of the options which requires high land areas devoted to specialist needs. If an option which entailed ponds or greenhouses were considered, then only part of the output can (practically) be employed. Under these circumstances, the use of part of each of the fractions of the total slurry output would be feasible.

The most obvious observation that can be made on the work carried out on the utilisation of anaerobically digested animal wastes is that the research has no strength in depth. Of the unconventional applications, only microalgal production, due to a long history of use with similar wastewaters, can be considered a proven technique. For many of the others, considerable barriers exist to their foreseeable implementation. In the case of yeast, for example, work has been limited to laboratory scale trials, while the use of the liquor as a hydroponics medium is virtually untried. Without basic work to establish the biological efficiency of such applications, no attempt to assess the economic value of digested slurry can be made. It is therefore neccessary initially to focus on the technical, rather than the economic aspects of the proposed options.

Diagram 2.4.a. shows one of the major limitations in the work to date: no effort has been made to extract the protein rich, suspended solid fraction. Attempts have been made to utilise these solids by ensiling the liquid with animal feed, but this use of the protein is at the expense of the nutrients in solution which may be suitable for a different application. Furthermore, unneccessary moisture is added to the ration. If the

suspended solids were removed from the liquor, a residual liquid, containing plant essential nutrients would be left. The obvious candidates for the use of this liquid are high rate microalgae production, yeast, or the hydroponic growth of a high value horticultural crop, such as tomatoes.

The initial work on hydroponics reported in **Appendix III** , indicated that the main problems arising from the use of the liquor were the presence of suspended solids, rendering pH control difficult, and the high BOD of the liquid. The removal of these solids may go some way to improving the potential of hydroponic plant production.

The main motivation for using the slurry is to exploit the changes in characteristics brought about by digestion, for economic gain. Under these circumstances, good candidates for this are the production of a high value horticultural crop, and the extraction of the single cell protein. It appears that the isolation of the microbial protein, and the production of a hydroponic medium may be achieved in a single step, by employing a separation technique. There are, therefore, good technical reasons for pursuing these options, if a separation technique appropriate for this application can be adopted.

Table 2.4.b. also indicates that nitrogen utilisation (as indicated by the estimated primary N recovery) increases as the method of food production becomes more complex. Where the waste is a direct input into a complex production system, such as open water fishculture, losses in efficiency (as defined by crude protein output) are incurred due to the sinks encountered at each trophic level. This may have an impact on economic performance. Within this framework, the optimal food production system is one which reduces the number of trophic levels involved. This goal may be achieved by using technology to prepare a material which is directly utilisable by the useful species of plant or animal that are being farmed.

2.4.2. General.

There are other reasons than economics for using the digested slurry effectively. Wilson and Brigstocke (1977) have pointed out that of the energy consumed by British Agriculture, 23.0% is on fertilisers. Morris et al. (1983) point to the energy expense of feeds, particularly concentrates, and suggest that a reduction in support energy input per unit of concentrate is desirable. More effective use of the slurry (by, for example, the extraction of the microbial protein) is beneficial in terms of the energy balance of developed world food production. Having said that, it must, however, be emphasised that only 4% of primary energy consumption is taken up in agriculture, with a further 12% taken up in food production.

On a global scale, efficient use of nutrients is desirable as the easily extractable deposits of phosphate are strictly limited (Wells, 1975), and fertiliser nitrogen is manufactured with energy from rapidly depleting fossil fuels.

Slesser (1973) makes this point on alternative ways to view the problem in some comments pertaining to unconventional protein production:

> "Economic analysis is associated with a set of values based on shortage, as perceived by participants in the market place. Perception of shortage is itself a recognition of supply and demand for the present instant and some time into the future. It gives little guide as to whether in the long term these perceptions are correct are not."

While such issues are important, they are rarely adopted as criteria for the selection of the most appropriate food production option. Neither the energy cost of food production, nor efficiency of fertiliser usage, are likely to be acceptable objectives in mainstream, developed world agriculture, the goal of which is to maximise economic returns.

Finally, the emphasis in examining the options has been on those in which some pretreatment is required. Some mention must be made of those applications in which the slurry may be applied direct. Some integrated agricultural systems have been constructed on the basis of a single input of unprocessed animal waste. These can be simple, but extremely elegant multifaceted food production systems giving rise to several protein products (Delmendo (1980) describes an operational system involving pigs,

open water fish culture, aquatic macrophytes and ducks). Such systems do not require controls as they are complex ecosystems which have built-in pathways through different trophic levels for nutrient and carbon release and reutilisation. The advantage of these systems is obvious- the system itself carries out the desired transformations. There are, however, limitations. As the system is essentially self regulating, efficient use of nutrients and carbon need not occur, and it is vulnerable to catastrophe, such as may occur in empirically based open water, manure fertilised, aquaculture.

Conventional fertilising with animal wastes is unique in this respect, being exceptionally stable. This is largely due to the ratio between applied organic material, and the organic pool in the well buffered biological and chemical system onto which the slurry is applied. It is axiomatic that the higher the existing system:added material ratio in terms of carbon and nutrient additions, the lower the propensity for perturbation due to that addition. Other outside influences may come into play, notably run off and subsequent eutrophication of open water, but all else being equal, and given the existing state of knowledge of other manure fed systems, land spreading is the most reliable option of direct application.

3.1. Introduction.

Chapter 2 has argued that of the strategies which could be adopted for the use of digested slurry, one of the best options is refeeding the protein rich solids to animals, in order to improve the efficiency of protein production, a desirable end in an animal-based agricultural enterprise. One of the main points to emerge from a discussion of the refeeding work to date, is that the separation techniques employed were inappropriate for the production of feed material. Coarse solids and ash have a low refeeding value, and are best rejected for this purpose. Thus a separator which captures the coarsest 60% solids of a slurry will effectively be achieving a negative material selection.

As the separation technique which is employed has such an influence on the value of the resultant material, it is appropriate to discuss those separators which are currently in use.

3.2. Filtration-Separation Techniques.

3.2.1. Separation Techniques in Agriculture.

The separation of animal slurries in solid and liquid fractions is commonly carried out for management purposes, and a range of devices is readily available "off-the-shelf" for farm applications. What is of particular interest here is the efficiency of separation, the ability to remove fine solids is of primary importance.

Most workers engaged on refeeding used centrifuges. This may have been a contributory factor in the lack of success. Hashimoto, Prior and Chen

(1978) used a bowl centrifuge at 5200 rpm and recovered a maximum of 35% total solids, with 23% of total organic nitrogen, from digested cattle slurry. Pfeffer (1977) using a bowl centrifuge at 3000 rpm achieved only 28-30% of total solids from digested cow effluent, and concluded that only marginal yield increases would be achieved at higher speeds. Furthermore, Pfeffer and Quindry (1981) showed that up to 60% of centrifuge captured total solids (of fermented corn stover) will be non-volatile (i.e. ash), and thus may present problems in refeeding.

The most promising separators, in terms of practical utility,Shutt et al.(1975) suggest, are stationary and vibrating screens, liquid cyclones and settling tanks. There is some agreement with this view from Hills and Kemmerle (1981), who prefer vibrating screens, sedimentation tanks, and sand filtering beds, while actively rejecting vacuum filters, belt filter presses, and centrifuges, mainly for reasons of cost, although these separator options will remove approximately 50% of the solids in the slurry.

Of the recommended techniques, most work has been done on vibrating, or moving screen separators. Shutt et al. (op.cit.) achieved the best separation performance (in terms of solid yield) with a vibrating screen, giving a total solids capture of 18.7%, and total volatile solids of 42.8%. This apparently low yield is not unusual: Shirley and Butchbaker (1975) with work on a rotating conical screen separator achieved similar capture values. Miner and Verley (1975) with a rotating flighted screened cylinder managed to obtain only 54.1% of the solids which were greater than 1.19mm in diameter, from feedlot waste. Gilbertson and Nienaber (1978) managed only 24±8% of total solids removed with a vibrating screen separator with a 20 mesh screen on cow solids. They also found that reducing the mesh size reduced filter capacity to the point where inflow could not be controlled. Hashimoto et al.(1978) also using a shaking screen achieved 50% of total solids, and Hills and Kemmerle (op. cit.) reported 60% solids reduction in digested cattle slurry (accounting for 35% of total nitrogen).

This (at best) 60% total solids removal is due to particle size distribution. As is discussed in **Appendix I** , and pointed out by Avnon (1980) and Pfeffer (op.cit.) among others, a definite break in particle

size of both cow and pig slurry occurs at about the 80-100 mesh size. While the capture of material larger than this is relatively easy to achieve, the suspended solids smaller than this are less amenable to removal from the liquid phase due to a suite of physico-chemical characteristics (Kavanaugh et al.,1978), the most important of which is their existence as a stable colloid.

Of the other options suggested by Shutt et al (op.cit.), the liquid cyclone gave 26.5% of total solids as a best performance, and the settling tanks, besides being (of neccessity) slow had an efficiency less than the other methods, on the basis of total solids removal. Hills and Kemmerle (op. cit.) echo this inefficiency, demonstrating only a 2% difference in total solid percentage between the upper and lower layers of a sedimentation tank over 48 hours. However the relatively high viscosity of a 6.5% solids content digested cow slurry would be unlikely to give a better result. Dilution of the slurry may have generated a higher degree of separation. The best results they obtained were the removal of 80.2% (volatile solids) using a sand bed, but problems were encountered in removing the material (without which clogging would rapidly occur) without contamination with sand grains.

Other active separation methods which have been attempted include vacuum filtration, which apart from being costly gives a low cake yield (Backer et al.1973), and pressure filtration which Bartlett, Bos and Wung (1975) found to cause the compression of the slurry forming an impervious cake, which neccessitated frequent stops. Menear and Smith (1973), using a screw press achieved a 21% organic matter yield when cycling undigested cow manure through the separator twice.

3.2.2. Other Separation Techniques.

Some attempt has been made to solve the problem of fine solid extraction. Ward, Johnson, and Keinholz (1975), using the "Cereco" technique, derived three fractions from feedlot manure; a high fibre silage obtained by screening, a high ash fraction by centrifuging the reject liquor, and a dried protein product, obtained by drying the supernatant from centrifuging. The dried protein product had a crude

protein content of 23.8%, but considerable ash remains (33.5%). This may partly explain the results of a feeding trial, in which over 30 days, when substituted on an isonitrogenous basis, the protein product failed to achieve the performance of a soybean control. This work highlights an important point: that more than one separation is required for protein removal, although a sensible compromise in terms of practicability and economics is needed. Senior's (1975) proposed method involves at least 10 process stages, including the use of brine and two flocculants to remove the protein rich fraction from beef manure. The complexity of this technique is such that it precludes its use on farms. (it has, furthermore, yet to be validated in terms of the suitability of the product as a feed material).

An interesting attempt to recover protein from animal waste has been made by Kravets and his coworkers (1980), by using the colloidal characteristics of the protein rich liquor using electrolysis, which releases protein (and oxygen) at one terminal, and hydrogen at the other. While being very energy expensive, it demonstrates the possibilities opened up by exploiting the chemical properties of the colloid.

Another such method is the use of flocculants, which cause the collapse of the colloid, and allow particle collection by one of the conventional separation techniques. Pfeffer and Quindry (1981) achieved significantly higher solid yields on digested corn stover with the addition of hydrated $FeCl$. This was only achieved on a bench scale vacuum filter, however. Prior and Hashimoto (1981) used the polyelectrolyte Magnifloc to increase nitrogen recovery from digested cow slurry to 60% (total, or organic is not specified), but have not conducted feeding trials with the product due to concern over harmful effects due to the flocculant, an anxiety shared by Lizdas, Coe and Turk (1981). Boersma et al.(1978) point to the expense, reduced digestibility and palatability of the flocculated product, together with toxicity effects.Hasdai and Ben-Ghedalia (1981) used alum as a flocculant for Chlorella, subsequently incorporated into the diet of sheep. Reduced P uptake (6.67%, compared to 29.5% for a soybean meal control) was attributed to the presence of alum. They comment that alum not only interferes with P adsorbtion, but will also cause severe depletion of body P reserves and that future methods for microalgae

harvesting should concentrate on producing a low mineral, low alumina biomass. While being specifically concerned with microalgae harvesting, this work points to possible drawbacks in the use of flocculants.

Furthermore, doubts about the efficiency of flocculants for use on digested animal slurries have been expressed. Pfeffer, whose background is sewage engineering, in comments on Stanton's (1980) conference paper, estimates that the conditioning difficulties are 10 to 100 times greater for digested cow manure than for digested sewage, while Hobson (1983) points to the relative thinness of digested animal slurry. Crocker (pers. comm.) failed to achieve flocculation of digested cow slurry with 15 types of additives.

In **Appendix** I , the relationship between crude protein content and particle size shows that larger particles have a relatively low crude protein content, compared to the smaller, suspended solids. The experience in animal refeeding of digested solids indicates that the high correlation between digestible protein and crude protein established for undigested material does not hold for the coarser solids of digested material. This is due to the loss of nitrogen associated with the plant derived material, with a concomitant gain in microbial protein, during digestion (Badger, Bogue and Stewart, 1979). The nitrogen which is left in the coarse solids is largely unavailable for short term microbial attack.

Conventionally employed separation techniques capture the coarser fractions of the solids and ash, which are limited in value for refeeding. This is not suprising, as they were designed primarily to ease routine animal waste management problems; namely the production of stackable solids, and the ease of pumping of liquids (Pain, Hepherd and Pittmann,1978). The protein rich solids are left in the residual liquor. To maximise the value of digester effluent as a feed material (specifically as a feed concentrate) the single cell protein must be removed from suspension, and separated from the coarse solids and ash. With the exception of two of the filtration techniques discussed above, which must be excluded due to either high energy consumption (Kravets, 1980) or complexity of operation (Senior, 1975), none are able to satisfy these criteria.

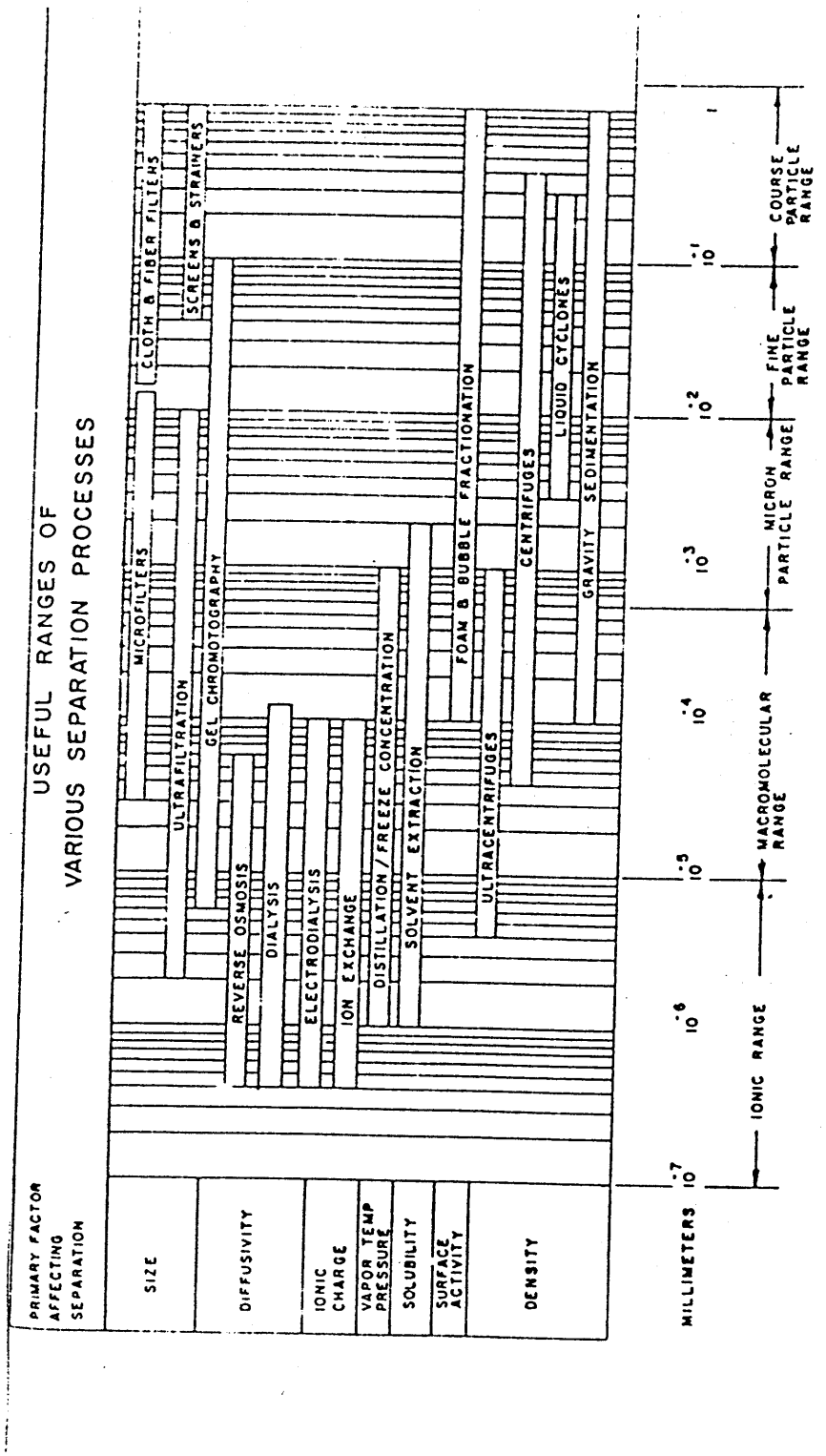
3.3. Alternative Separations.

In the previous section it was argued that conventionally employed separation techniques are unable to achieve the desired complete solid removal from the effluent. However, removal of the solids may be achieved in one of two ways: by increasing the sophistication of the separation, or by effectively increasing the solid size. There are many effective techniques for fine solid removal. Diagram 3.2.a. (after Conway and Ross, 1980), shows the range of options, together with an estimate of the lower size limit. With increasing sophistication, however, goes increasing cost, both running and capital. As the intention is to maximise the economic potential of the effluent in food production terms, such a solution should, if at all possible, be avoided.

Alternatively, the problem may be solved by increasing the solid size. The chemical route for achieving this (by the use of flocculants) has been discussed and rejected for reasons of the difficulty of conditioning the liquid, and the possible toxic effects in animal feeding. Particle flocculation may be achieved by certain aerobic treatment processes utilised by the sewage industry. Grady and Lim (1981) indicate that soluble organic waste removal, and nitrogen transformation may be achieved by three generic biological wastewater treatment processes-trickling filters, rotating biological contactors, and activated sludge systems. In activated sludge treatment, flocs of bacteria form at solid-liquid, or liquid-air interfaces, and are removed from the liquid by settlement, or by a conventional filter press. In the case of the rotating biological contactor, flocs form on the polythene discs which cut the surface of the effluent pool, and are sloughed off by the mechanical action of the disc moving through the liquid. These solids are collected in a sedimentation tank. For trickling filters, solids are removed by periodic backflushing of the entire pond or tower (Conway and Ross, op. cit.).

Aeration has been used quite extensively as a method of treating animal wastes as a preparation for refeeding. Harmon and Day (1975) used an oxidation ditch for stabilising pig effluent for use in direct refeeding, and Hegg et al.(1974) fed aerobically treatedbeef cattle manure. More recently, Martin and Loehr (1983) have used aerobic stabilisation of

Diagram 3.3.3.a. Useful ranges of various separation processes. (from Conway and Ross, 1981).



laying hen manure to produce a feedstuff, and demonstrate improved quality, notably in essential amino acid content.

Aeration is also appropriate for the production of a liquid suitable for hydroponics. The problems encountered when using the digested liquor directly for NFT were partially overcome by direct aeration of the recirculating liquid (appendix III). A formalised aeration process before direct use may not only achieve the goal of generating settleable solids for use as an animal feed, but will reduce BOD , solids and ammonium nitrogen contents (Arceivela, 1980), and remove phenolics (Garraway and Ramirez,1982), factors which were considered to be the major problems in using the digested liquor for hydroponics.

3.4. Aeration.

It appears that aeration may be a suitable vehicle for the extraction of the proteinaceous suspended solids, leaving the aqueous phase a medium for hydroponic plant production. Aeration is, however, a generic process with many variations, and a technique which answers the specific needs of this separation is required. One of the lessons learnt from the application of conventional separation techniques to digested animal slurries for the production of a feed material is that the separator may, or may not be appropriate for the task of isolating that fraction of the solids which is best suited to as a feed, simply because it fulfills the condition of removing some of the solids in the slurry. In order to avoid the possibility of employing an aeration technique which does not satisfactorily produce both a liquid and a solid for further food production applications, some initial criteria for the aeration system need to be established.

3.4.1. Nitrogen Conservation.

The use of aeration in a system whose primary aim is the conservation of nutrients (and thus the maximisation of output per unit of raw slurry) raises problems. Aeration is most frequently used in the sewage industry. These wastewater treatment systems enhance nutrient losses, notably

nitrogen, by ammonia volatilisation, and occasionally by active nitrification-dentrification processes (Eckenfelder, 1980). This is a reasonable position for an industry whose remit is to 'clean' water. In agriculture, however, the priorities are different.

The literature on the subjects of livestock waste management, and landspreading of those wastes, reveals a mismatch between attitudes towards the material during, and after processing. Conservation of nitrogen is considered to be a primary objective in land spreading, and careful attention is paid to timing, and the mechanical mode of spreading or incorporation (Gracey,(1977) and Terman (1979) as examples). However, the management of the problem immediately after production focusses mainly on the containment, or reduction of the pollution potential (Grundey,1980). This is often at the expense of nutrient conservation.

Both of these objectives should be primary considerations all the time, in order to conserve an energetically and economically valuable resource; and control pollution. The links between the aims of agronomists and agricultural engineers in this context appear tenuous, and aerobic treatment systems used in agriculture normally result in high nitrogen losses (Owens et al.(1973); Vanstaen et al.(1976); Loynachan et al.(1976); and Garraway (1982) as examples). As both the aqueous phase and solid organic nitrogen are considered resources in this work, the aeration process should inhibit nitrogen losses.

3.5. Conclusions.

The problem of validating a separation technique in terms of the utility of the product has already been mentioned. In the context of this work, the most important criterion of a successful separation is the suitability of the product(s) for use in food production. The methodology adopted for the development of a separation technique appropriate to these needs may be summarised as follows: the development of an experimental system for the generation of the solid and liquid nutrient sources; application of these materials to the biological systems under investigation and, following a successful application, improvements in the efficiency of the separation. The process is neccessarily iterative. As

this is the case, the experimental aeration equipment used here is simple- a batch system whose primary purpose is as a vehicle for the production of material for *in vivo* testing of the proposed food production systems. Some attention must nonetheless be paid to the efficiency of the aeration technique.

4.AERATION.

4.1. Introduction.

It has been stated that the requirements of the aeration process are the removal of BOD and suspended solids, oxidation of soluble organic compounds in the slurry, and transformation of ammonium nitrogen. The three aeration techniques which carry out these transformations on effluent waters are trickling filters, rotating biological contactors (RBC's), and activated sludge systems. The solids produced by all three processes are similar, comprising aerobic bacteria, particulate solids from the effluent, and bacterial bi-products. For RBC's and trickling filters bacterial accumulation on inert surfaces is brought about by the sucessive processes of the transport and absorbtion of organic molecules to the clean surface, the transport and adhesion of microbial cells to the conditioned surface, followed by the steady state of continued transport and adhesion of microbial cells to the established biofilm, together with secondary microbial growth. Nutrients and metabolites diffuse in and out of the biofilm (Characklis, 1981).

The action of particulate flocs in activated sludge systems is a special case of biofilm formation- the processes of solid accumulation, nutrient uptake and metabolite production are the same. Flocs of bacteria are provided by recycling previously "activated" sludge. The build up of such flocs may be achieved without recycling treated effluent, but requires the conservation of the slurry of naturally flocculent bacteria, and contemporary activated sludge systems rely on continuous cell recycle (Grady and Lim,1981). In other respects, however, removal of soluble and particulate organics is achieved in the same way. For activated sludge, the flocs form in the bulk liquid, while for the RBC a biofilm forms on the inert (plastic) rotating discs, but due to the mechanical action of the system, is sloughed off and collected in a settling tank. The trickling

filter has a biofilm which forms on the inert medium, containing the active microbial population, and is essentially a fixed bed reactor. When excess build-up of the biofilm occurs, causing clogging, the entire system is backflushed.

From the standpoint of ease of collection of the microbial substrate following aeration, a trickling filter is preferable, as the solid may be removed from the inert substrate, whereas the solids from the RBC and activated sludge systems require an active separation mechanism, being held (although not colloidally) in the aqueous phase.To this end, a trickling filter was constructed to provide the materials for trials in the proposed food production systems.

The most obvious problem with the selection of this technique is that the inert medium on which the biofilm forms normally comprises either rock fragments, or plastic media resembling egg crates (Grady and Lim, op. cit.) in order to increase the surface area of the medium per volume of filter (as the surface area describes the reaction rate). Such shape configurations render the collection of solid difficult. To circumvent this problem, a trickling filter with planar surfaces was employed (at the expense of efficiency).

The experimental work on aeration is divided into two parts, each relating to separate attempts to generate both a solid and a liquid suitable for reuse in food production. Because of its iterative nature, this work is reported and discussed sequentially.

4.2.Aeration, Part 1.

4.2.1.Introduction.

This report focusses on the initial attempts to generate a proteinaceous feed material from the liquor. The first iteration of the trickling filter is described. In addition, efforts to make use of another mis-placed resource, waste paper, are reported. As paper potentially has a feed value, it was used as the inert material which provides the key for

biofilm development.

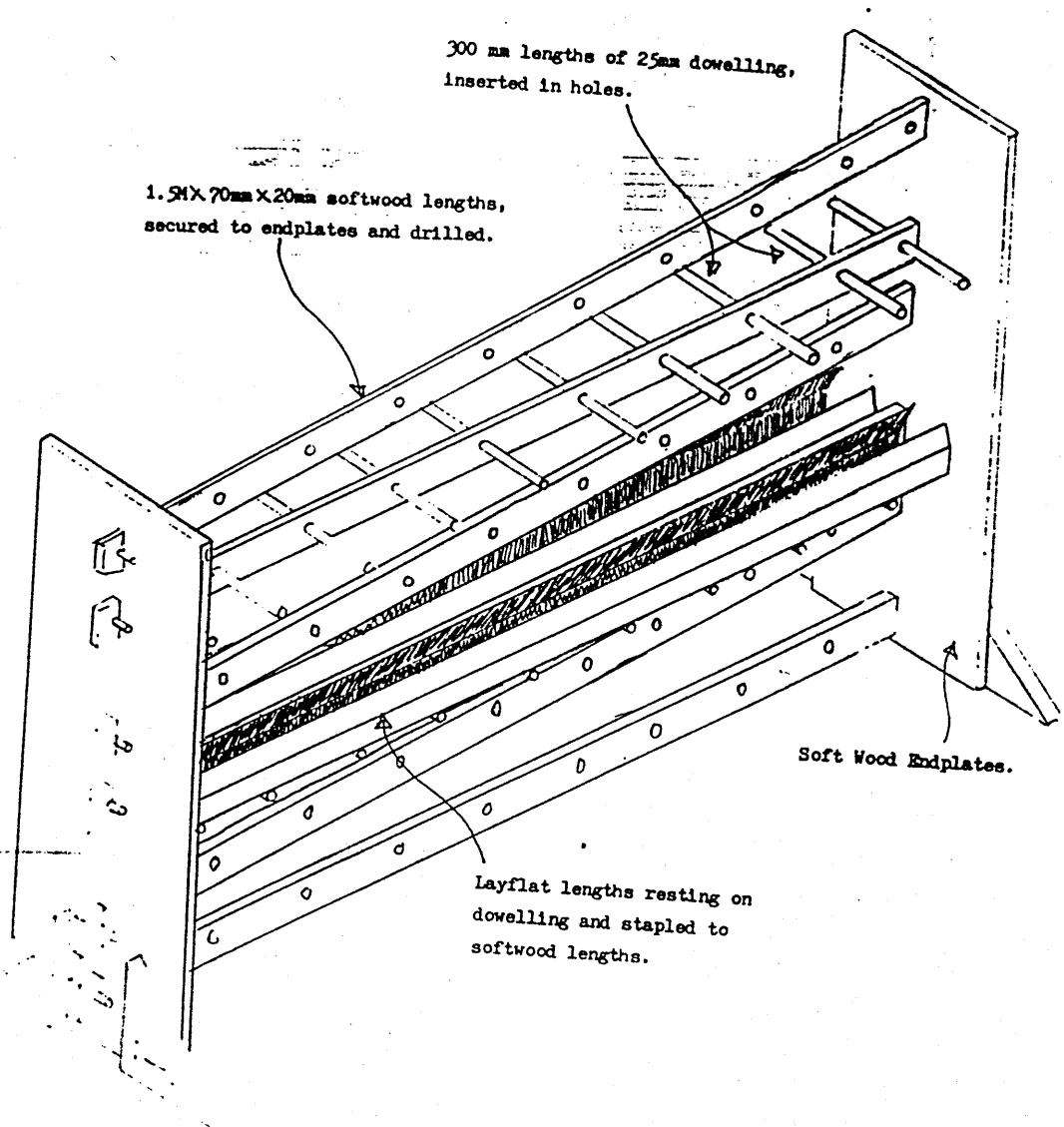
4.2.2. Paper.

The feeding of paper to animals, notably ruminants, is a common practice. There are, however, quality limitations to its usage. There are two basic papermaking techniques, varying in chemical pretreatment and final content. Mechanical pulping involves beating and washing the raw material prior to paper formation. In this technique, no lignin removal is carried out, but almost all the water soluble hemicelluloses are lost.'Groundwood' paper, as this is known, contains 55-65% cellulose, 15-25% lignin, 1-3% ash, and low levels of lipid and protein. Chemical pulping brings about delignification by cooking the pulp in a sulphite, sulphate or sodium hydroxide solution, followed by beating or washing of the fibre. This paper contains 90-95% cellulose, and low levels of lignin and ash (Dunlap,1980).

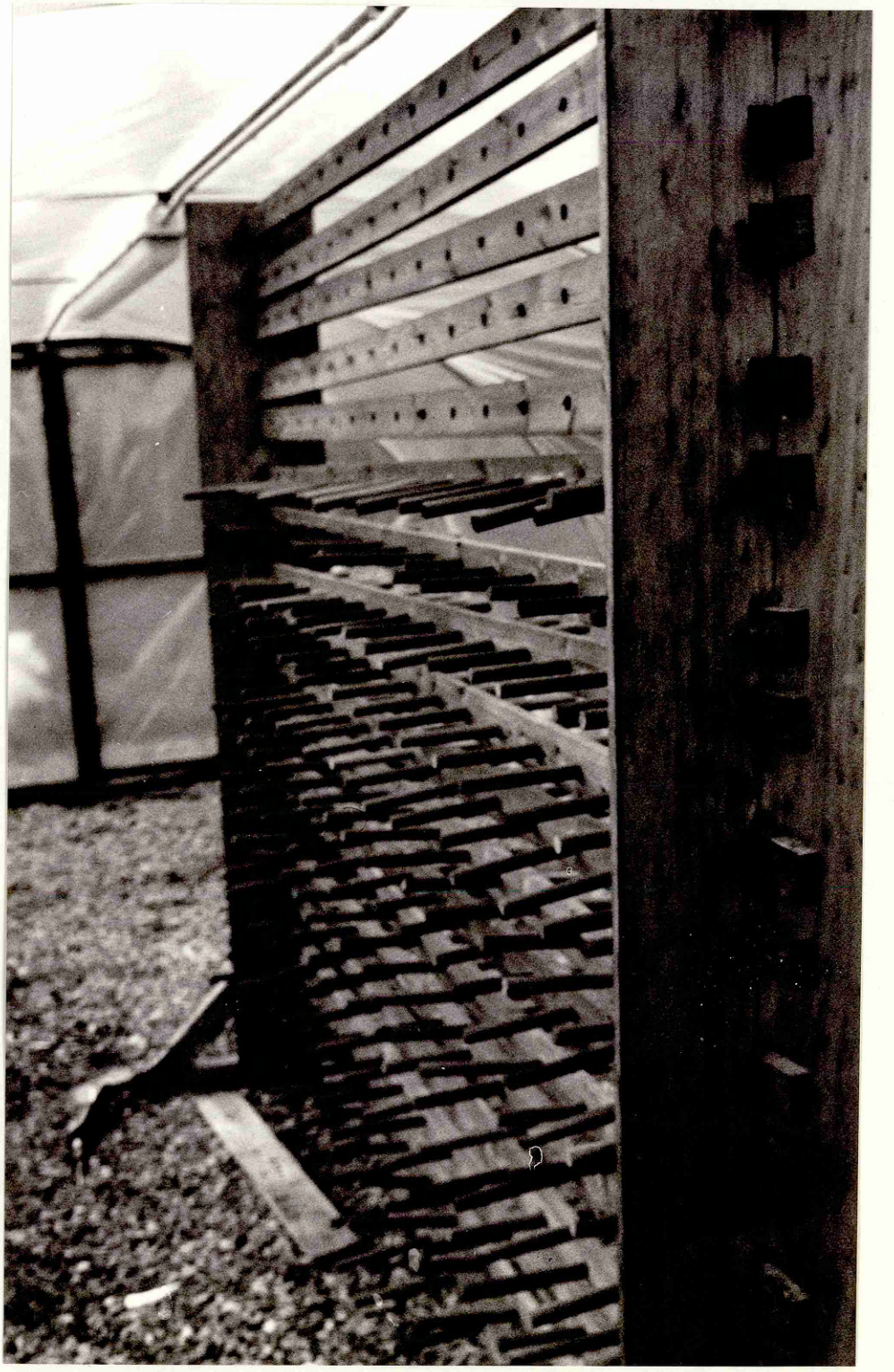
The high lignin content of groundwood paper inhibits good fibre formation (Robinson,1981), and is used for low quality purposes, such as newspaper. Chemically pulped paper is commonly employed where intrinsic strength or quality is important, such as wrapping and writing papers. Admixtures of the two pulps are used depending on the required quality of the product, and consequently the lignin content is variable. Gillies (1975), in a review of paper feeding to ruminants, concludes that in vitro digestibility improves with lowered lignin content. The low digestibility of high lignin content papers may be improved by ensiling, or soaking in an alkaline solution. The fragility of the paper precludes the use of normal soaking techniques, however.Soaking paper under the conditions of the filter may alleviate this problem.

4.3.Materials and Methods.

Diagram 4.3.1.a. Three Dimensional Representation of the first Experimental Filter, showing progressive construction stages. The slurry sump, pump, and tubing are not shown.



Photograph 4.3.1.a. Showing the filter approximately half constructed, detailing the wooden support.



4.3.1.The filter.

Diagram 4.3.1.a. gives a representation of the filter used in this experiment. It comprises 15 lengths of softwood, 1.5MX70mmX20mm, through which, at 150mm intervals, on the 70mm face, 25mm diameter dowelling lengths, 300mm long, have been driven, such that the dowelling protrudes equally from either side of the pine length. Each pine length is secured to two end plates of soft wood, such that a fall of 1 in 50 is experienced from one end to the other. Alternate lengths are secured so the fall is in the opposite direction to the one immediately above, and below. 1.5M lengths of NFT layflat(see Appendix III) provide the planar channelling, and rest on the dowelling protruding from each side of the pine length.The layflat is secured to the pine length by staples. A vertical clearance of 150mm from the end of one length, and the start of the next, was maintained. The lowest lengths of layflat drained into a 250 litre plastic sump tank which was buried in the ground. Photograph 4.3.1.a shows the filter at a stage approximately half way through construction, indicating the vertical dowelling and the softwood lengths.

4.3.2.Experimental.

The layflat lengths were lined with strips of soft paper, and the sump tank filled with 150 litres of that fraction of digested pig effluent which passed a 1mm sieve. Slurry was pumped to the top of the filter via a 190W Beresford pump, and 25mm i.d. PTFE pipe, into the top end of which were spliced 2 5mm feeder pipes, one for each 'run'. Slurry supply was regulated at 500cm^3 minute^{-1} run^{-1}. Flow regulation was achieved by a bipass above the pump, the excess flow being redirected into the sump via a 25mm pipe fastened above the surface of the slurry, such that the returning flow broke the surface and afforded some aeration.

Samples were taken daily and total solids, pH, and total nitrogen determined. The slurry in the sump was replaced three times and subjected to the same treatment, except that on the third occasion a polythene tent was erected around the filter. The paper was kept <u>in situ</u> for the three runs, but finally removed, air dried, and placed in a dessicator. Solid accumulation per unit surface area,(determined by weighing measured areas

Table 4.4.1.a. Collected Measurements taken during the aeration experiemnts with the first aeration tower.

	Run 1.			Run 2.				Run 3.(polythene cover).			
Day	0	1	2	3	0	1	2	3	0	1	2	3
pH	8.7	9.1	8.6	7.8	9.1	9.0	8.0	7.7	8.7	8.7	8.1	7.3
Total N* (mg/L)	1382	2880	4160	5025	1559	1440	3860	4260	1408	1629	3390	6425
Total Solids (%)	1.07	2.18	3.29	5.66	1.22	1.44	2.24	4.84	0.98	2.86	3.91	4.37

* In liquid.

of paper), total nitrogen content were determined. A sample of the paper extract was sent to ADAS at Wye for amino acid analysis.

4.4.Results.

4.4.1.Filtration.

Table 4.4.1.a. shows the measured parameters for the three runs. In all cases the run time lasted only 3 days, due to the high evaporative losses experienced. In each case, at the end of this period, the sump tank had insufficient slurry to be pumped. There is no apparent difference in evaporative loss due to shrouding with polythene.

The total solid content of the liquid increases due to evaporative losses, so that indications of solid removal from the bulk slurry are impossible to detect, by measurements of the liquid. There is a marked fall in pH, after an initial period of stasis, or small rise on the first day.

4.4.2. Paper.

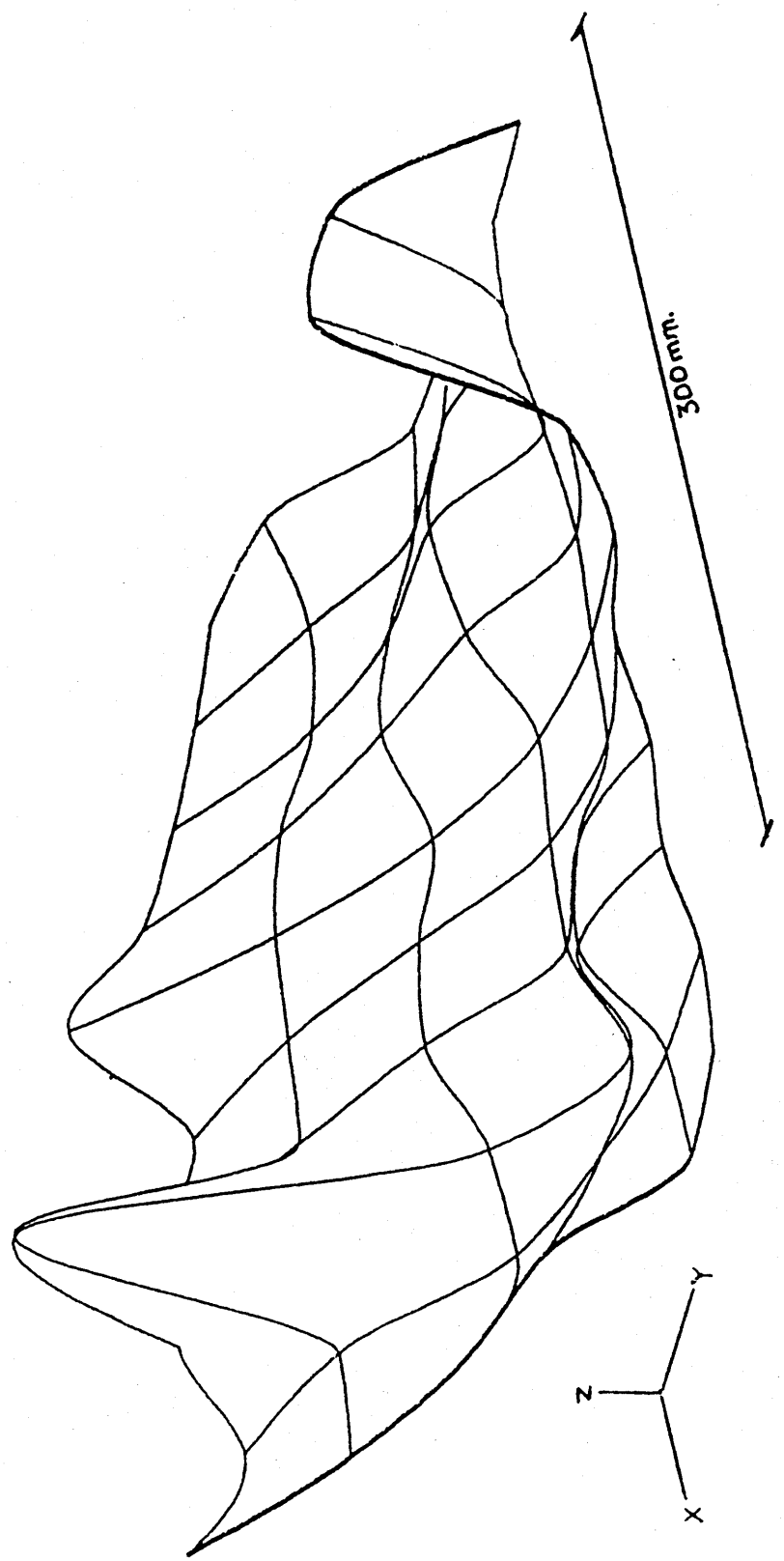
Solid accumulation on the paper did occur, but a large variation in solid accumulation on the paper base was noted. Diagram 4.4.2.a. shows a three dimensional representation of solid accumulation, at 10mm intervals, for a sample section of paper .12M×.30M, indicating that biofilm development is highly variable. The mean solid accumulation of the sample paper is 34g $\bar{M}^2$ of surface area, with a standard error of the mean value of 20g $\bar{M}^2$.

4.5.Discussion.

4.5.1.General.

It is apparent that in terms of generating both solid and liquid for use as a feed material, and hydroponic medium, the experiment was not successful. With complete evaporative loss of the liquid, and irregular

Diagram 4.4.2.a. A three dimensional representation of solid accumulation (z axis) on a paper sample removed from the filter. (Flow direction, x axis).

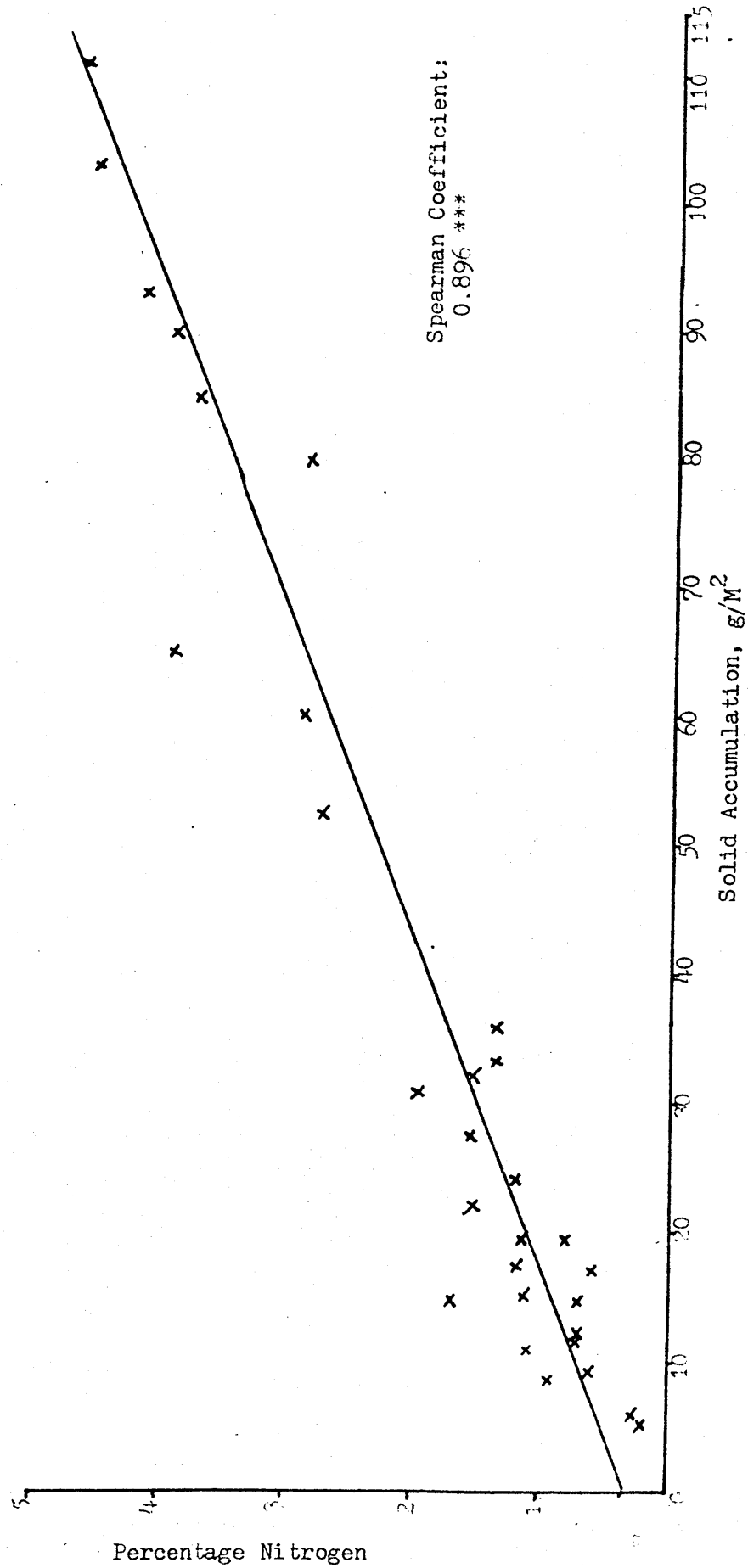


45a

45a

3 DIMENSIONAL REPRESENTATION OF BIOFILM DEVELOPMENT IN ROUGH FILTER TILE, FLOW DIRECTION L to R.

Graph 4.5.4.a. Regression of solid accumulation (g M^{-2}) against percentage nitrogen of the paper product.



solid accumulation, very little is accomplished.The technique adopted would be useful if slurry disposal, rather than utilisation, were desired, however.

4.5.2.Aeration.

The initial rise in pH observed in the first run, and the plateau on day 01 noted for the second and third runs, is consistent with other observations made for the aeration of piggery effluent (Vanstaen et al,1976). The subsequent fall in pH is due to ammonia volatilisation. The apparent increase in solid content and total nitrogen in the slurry is due to the evaporative losses experienced over the experimental period.

4.5.3.Paper.

The potential of the paper-based product as a feed material depends on the reliability of the product.Diagram 4.4.2.a. has demonstrated that biofilm development is highly variable. In an application where the resultant material needs to be finely comminuted, and incorporated into a diet on an isonitrogenous basis, low variability is required. The material is unsatisfactory in that respect.

Graph 4.5.4.a. shows the relationship between solids accumulation, and nitrogen content. In order to derive a material with a sufficiently high crude protein content, careful selection of the paper with high solids accumulation was carried out, on the basis of paper colour. The crude protein, and essential amino acid content, are given in table 4.5.4.a. Despite the intrinsic variability of this product, the crude protein content was considered sufficiently high to use in a feeding trial.

5.Conclusions.

This work is an attempt to bring together the themes of solid and liquid separation, aeration, and the use of waste paper in a single experiment. It could be argued that these themes are too diffuse to be treated all together, and would best be looked at in a series of experiments. The unsatisfactory results would appear to bear this out.

Table 5.4.5.a. Essential Amino Acid, and Crude Protein Content of the paper extract.

Amino Acid.	Weight g. $100g^{-1}$ dry material.
Arginine	1.22
Histidine	.43
Leucine	2.34
Isoleucine	1.56
Lysine	1.38
Methionine	.15
Cystine	.45
Phenylanaline	.56
Tyrosine	.94
Threonine	1.27
Tryptophan	a
Valine	2.15
CRUDE PROTEIN (%)	23.0 (4.86)[b]

a Destroyed by acid hydrolysis.

b Standard Error of the Mean.

However, the logic of using paper, which has an intrinsic feed value, as an inert base can be justified. What is lacking in this work is insufficient consideration of the practical implications of bringing together these diverse elements in a single, somewhat speculative experiment.

Despite this, some useful points emerge: firstly, if the liquid phase is considered a resource, steps must be taken to reduce evaporative, and volatilisation losses. In addition, it is apparent that solid accumulation on planar surfaces does occur, so that the idea of this technique for the laboratory scale collection of solids for feeding trials may be pursued.

5.DIGESTED SWINE EFFLUENT AS A FEED MATERIAL.

5.1.Introduction.

This experiment is an attempt to evaluate the feed value of the paper-based proteinaceous material extracted from anaerobically digested piggery waste. This material is substituted for a conventional protein source in the feed of a monogastric animal. Fish are advantageous as they express mature nutritional habits at a small size. The relatively juvenile state of the the experimental population allows a rapid expression of the differences between feed materials. Common carp (Cyprinus carpio) were chosen as they only utilise true protein, and there are comparatively few problems in culturing this species.

5.2. Materials and Methods.

5.2. Feed Preparation.

The product from the aeration experiments was air dried, ground to a powder passing a 1mm sieve, and placed in a dessicator for 48 hours. It was then incorporated at 10% and 20% substitution levels for other protein sources on an isonitrogenous basis.Food preparation was by dry mixing of the ingredients, all of which had been ground to pass a 1mm sieve, followed by the addition of sufficient water to form a paste, which was kneaded for 10 minutes, and then extruded through a mincer with a 4mm die.

The threads of feed produced were dried using electric fans. The resultant feed was then ground to pass a 2mm sieve, and the fraction that was retained by a 0.5mm sieve was used. Particles smaller than that were recycled by wetting. In the latter part of the experiment the lower size limit was raised to 1mm., due to increased fish size.

Table 5.2.a. Percentage Contribution (by weight) of the dietary components in the three experimental diets, with estimated and measured protein contents, and fat contents.

Substitution Level:	0%	10%	20%
Wheat Middlings.	10	6	2
Soyabean Meal.	10	6.5	2.5
Brewers Yeast.	25	25	25
Fish Meal.	25	23.5	23.5
Skimmed Milk.	18.5	17.5	15.5
Protein Extract.	0	10	20
Cod Liver Oil.	3	3	3
Corn Oil.	2	2	2
Mineral Mix.[a]	1	1	1
Vitamin Mix.[b]	2	2	2
Chromic Oxide.[c]	.5	.5	.5
Binder.[c]	3	3	3
Crude Protein (nominal)	37.86	37.04	36.72
Crude Protein (measured)	36.81(.64)	34.22(2.18)	32.64(1.76)
Fat	8.01(.55)	7.78(.34)	6.27(.81)

a. Mineral Mix comprises:

Ingredient	g 100g^{-1}
Calcium tetrahydrogen orthophosphate	68.62
White Limestone	5.45
Magnesium Carbonate	9.06
Ferrous Sulphate	2.99
Potassium Chloride	4.98
Sodium Chloride	7.97
Aluminium Sulphate	.0264
Zinc Sulphate	.398
Copper Sulphate	.097
Manganese Sulphate	.27
Calcium Iodate	.0245
Cobalt Sulphate	.0997

b. Vitamin Mix comprises: The minimum requirements of Thiamine, Riboflavin, Pyridoxine, Panthotenic acid, Folic acid, Choline, Niacin, B_{12}, C,E,K, (as laid down by Tacon and Ferns (1976)) are met by "Sea Vita" of Aqualife Imports, Ltd., Heathrow. Inositol, and Rovimix H were added independently.

c. Carboxymethylcellulose, disodium salt.

Table 5.2.b. Essential Amino Acid Contents of the Diets (g A.A. . $100g^{-1}$ dry feed), compared to the Requirements of Carp.

Amino Acid	0% Substitution	10% Substitution	20% Substitution	Requirements.
Arginine	2.54	2.41	2.38	1.7
Histidine	1.09	1.05	1.03	0.8
Leucine	2.46	2.39	3.19	1.3
Iso-Leucine	2.26	2.20	2.18	1.0
Lysine	3.34	2.17	2.29	2.2
Methionine	1.39	1.21	1.27	1.2 or 0.8*
Cystine	0.51	0.53	0.55	0 or 2.0
Phenylanaline	1.62	1.85	1.87	2.5 or 1.3
Tyrosine	1.44	1.41	1.44	0 or 1.0
Threonine	1.91	1.89	1.91	1.5
Tryptophan	0.53	0.47	0.43	0.3
Valine	2.61	2.64	1.63	1.4

* The alternative values offered for these amino acids is because methionine and cystine, and phenylanaline and tyrosine, respectively, are interchangeable. Hence if the methionine requirement is set at 1.2, then no cystine is required, etc.

Table 5.2.c. Amino Acid Contents (g 100g^{-1} of dry material) of the dietary components.

Amino Acid	Wheat Middlings	Fish Meal	Soyabean Meal	Skimmed Milk	Brewer's Yeast	Protein Extract.
Arginine	.98	5.17	3.28	1.23	2.37	1.22
Histidine	.40	1.84	1.22	.92	1.19	.43
Leucine	1.22	5.69	3.99	3.54	3.46	2.34
Iso-Leucine	.79	3.43	3.11	2.34	2.32	1.56
Lysine	1.22	6.30	3.05	2.69	3.33	1.38
Methionine	.23	3.14	2.01	.96	.82	.15
Cystine	.25	.82	.57	.47	.55	.45
Phenylanaline	.72	2.99	2.34	1.69	1.96	.56
Tyrosine	.43	2.43	1.55	1.21	1.62	.94
Threonine	.59	3.15	1.89	1.69	2.25	1.27
Tryptophan	.24	.85	.69	.47	.55	a
Valine	.86	4.78	2.45	2.46	2.50	2.15

a destroyed by acid hydrolysis.

5.2.2.Feed Formulation.

Protein requirements for carp are relatively well established; Ogina and Saito (1970) have given a value of 380g. of protein per kg of feed, and Sin (1973a) has found that the highest protein content of fish occurred with a diet containing 38.4% protein, notwithstanding a slightly low metabolisable energy content in the diets. Consequently, a protein level of 38% was chosen.

Table 5.2.a. shows the percentage by weight of the diet components for the three experimental feeds, together with the nominal and measured protein contents, and the fat contents. Because the extract has a relatively low crude protein content, the main dietary component which is substituted is wheat middlings. This makes Brewers' Yeast the largest single dietary element. Such a formulation is generally avoided due to the high cost of Brewers' Yeast, and the presence of copper (K.Jauncey, pers. comm.). The material used (Yestamin, from Overseal Foods Ltd., Burton-on Trent), has only 6ppm Cu, and cost is, at this point, not an important consideration.

Table 5.2.b. shows the essential amino acid contents of the diet, together with the amino acid requirements for carp, from Nose (1979). The shortfall in Lysine content noted for the 10% substituted diet has been ignored as the lysine values employed were available lysine for pigs. In addition, a rounding up of the value to a single decimal place would satisfy the carp needs for this amino acid. Table 5.2.c. indicates the amino acid contents of each of the dietary components, taken from suppliers' specifications and feeding tables.

Table 5.2.d. shows the estimated metabolisable energy values of the diets, based on Stickney's (1978) data on the M.E. values of feed materials. All the diets meet the minimum needs, set by Sin (1973b) of 3000 kcal/Kg (12.6 MJ/Kg). As no information on the true metabolisable energy value is available, it has been assumed that the protein extract has a value of 2000 kcal/Kg (8.4 MJ/Kg), similar to that of alfalafa meal. This value is half the conventionally attributed value of 4000 kcal/Kg (16.8 MJ/Kg) for protein (Stickney, op. cit.)

Table 5.2.d. Estimated Metabilisable Energy Contents of the diets, by components and totals. (kcal. kg^{-1}).

Component	0% Subst.	10% Subst.	20% Subst.
Wheat Middlings.	260	156	52
Soyabean Meal.	220	143	55
Brewers Yeast.	1000	1000	1000
Fish Meal.	800	750	750
Skimmed Milk.	740	700	620
Protein Extract.	-	200	400
Cod Liver Oil.	246	246	246
Corn Oil.	198	198	198
Totals (kcal. kg^{-1})	3464	3393	3321
Totals (MJ. Kg^{-1})	14.49	14.19	13.89

5.2.3.The Aquarium System.

An open flow system, comprising 12 rectangular tanks with a holding capacity of 120 litres, stacked in 3 ranks of 4 high, was constructed. Each tank received water via piping fitted with a flow control valve controlling input, from a header tank. The header tank received mains water, after it had passed through an activated charcoal filter to remove chloride ions, and heated to 23°C by a commercial shower unit heater. Overflow water from the tanks was channelled, via circular ports cut into the tank ends approximately 30mm below the top of the tank, and piping, direct to the drains. Water flow through the tanks was maintained at approximately 250±50 ml per minute, assuming a complete water change every 8 hours. The tanks were strongly aerated.

5.2.5.Experimental.

Fingerling mirror carp (Cyprinus carpio), with an average weight of 4.5g, were obtained from Cotswold Carp Farm (Bourton on the Water). Twenty five randomly selected fish were placed in each tank, and allowed to equilibrate for 3 weeks on a home-made, extract free diet. The tanks were assigned to three treatments: control, 10% and 20% substitution with the extract, in a randomised block design.

At the start of the experiment the fish from each tank were collectively netted, allowed to drain for 10 seconds, placed in a tared bucket of water and weighed. No feed was given 18 hours before weighing. This weighing method was followed every ten days until the end of the experiment. In addition, three fish were sacrificed to establish initial protein content, in order to compute the the protein efficiency ratio of each of the diets.

The fish were fed at 4% of body weight daily on their respective diets, the feed conversion ratio from the previous weighing being used to predict weight increases over the 10 day period. The level of 4% was selected, as it is common in such trials, notwithstanding Huisman's (1976) indication that this value is sub-optimal.

At the end of the experiment 3 fish from each tank were sacrificed to establish fat, ash, and nitrogen contents. Nitrogen was determined by a modified macro-Kjeldahl method (ADAS,1981), fat by the chromatography column method (Korn and Macedo,1973), and ash by heating in a muffle furnace at 550°C for 18 hours. Due to the low sample number statistical testing was by means of the Mann-Whitney U test, as recommended by the EIFAC(1981) guidlines on fish experimentation.

It was initially intended to determine digestibility by the method of Furukawa and Tsukahara (1966). Due to the premature cessation of the experiment, however, this had to be abandoned, as there was no certainty that any food had been consumed during the last few days of the experiment.

5.3.Results.

5.3.1. General.

The feed formulation method was satisfactory. The pelletised feed material did not break up, and was acceptable to the fish.

5.3.3. Growth and Protein Utilisation.

The average relative growth values of the three treatments (indexed from 100) are shown in graph 5.3.a., while the specific growth rates are indicated in table 5.3.b. The control treatment gave the highest growth performance, both in terms of relative growth, and specific growth rate, which was significantly greater ($P<0.05$) than both the substituted treatments. This trend was followed in the feed conversion ratios, the control diet being significantly lower than the other treatments.

The results are less clear for the indices of protein uptake and use (Table 5.3.b.). No significant difference for any treatment was detected for apparent net protein utilisation (a measure of nitrogen retained, against nitrogen consumed), while the protein efficiency ratio (the relationship between weight gain and protein intake) was significantly lower for the 10% treatment, compared to both the control and 20%

Graph 5.3.a. Mean Relative Growth for the control and Substituted Treatments, over time (indexed from 100).

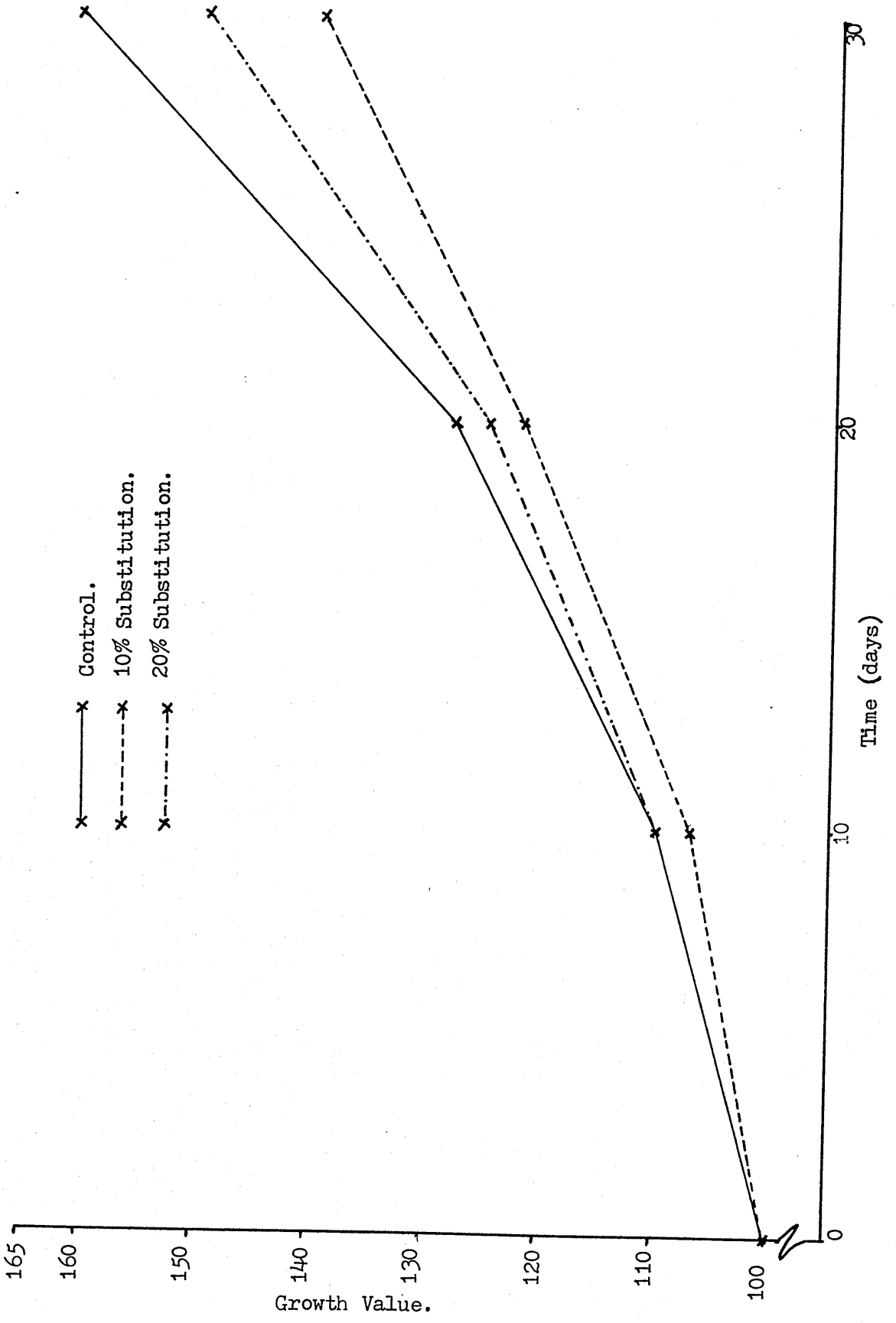


Table 5.3.b. Overall Growth and Protein Utilisation Parameters for the three diets.

Parameter	0% Subst.	10% Subst.	20% Subst.
Feed Conversion Ratio.	2.16(.09)*	3.15(.37)	2.73(.34)
Specific Growth Rate.	1.84(.12)*	1.17(.15)	1.28(.15)
Apparent Net Protein Utilisation.	.54(.12)	.35(.15)	.37(.11)
Protein Efficiency Ratio.	.90(.07)	.68(.07)*	.85(.08)

Brackets indicate standard errors of the means.

* indicates the value is significantly different ($P<0.05$) within rows.

Table 5.3.c. Proximate Analysis of Harvested Fish, for the three treatments.

	0% Subst.	10% Subst.	20% Subst.
Protein.	54.36(5.85)	50.45(4.88)	47.85(3.27)
Fat.	9.69(1.11)	13.59(0.23)*	8.52(1.31)
Ash.	13.12(0.51)	13.37(0.22)	15.91(0.59)*

Brackets indicate standard errors of the mean values.

* indicates a significantly different value ($P<0.05$) within rows.

treatments.

5.3.2. Carcass Analysis.

Table 5.3.c. shows the average protein, ash and fat contents for the fish analysed at the end of the experiment. No significant difference in protein content between the treatments was noted, while the ash content of the 20% substituted diet was significantly ($P<0.05$) higher than the other treatments. The fat content of the 10% substituted diet was greater than the other treatments ($P<0.05$).

5.5.Discussion.

5.5.1.General.

Due to problems with mains water quality (Anglia Water Authority were in the process of flushing the mains system with a permythrin based biocide), the experiment was prematurely terminated. The final feed conversion ratios (not shown here) were either negative, or in excess of 300:1, indicating the lack of feed uptake. The sample fish for proximate analysis were collected during this final weighing. This disruption has two main implications. The experiment may not have run sufficiently long for adequately large differences between the treatments to emerge (although the results from trials of 30 days duration have been published e.g. Tacon and Ferns,1976; Huisman,1976). In addition the proximate analysis of the fish may be unreliable due to the low feed uptake during the final 10 days, when body reserves would be used for maintenance, rather than growth. The index of apparent net utilisation is computed from the results of the fish analysis, and must therefore be treated with some circumspection. Growth and protein efficiency measurements are unaffected.

The higher performance of the 20% substituted diet, compared to the 10% substituted diet, is attributable to low available lysine (compared to total lysine) content in the soya bean meal. The content of lysine is higher in the 10% diet, compared to the 20% diet.

5.5.2. Protein Utilisation.

There is some debate as to whether common carp can utilise cellulose: Shcherbina and Kazlaukene (1971) have indicated that up to 50% of crude cellulose in the diet of (2 year old) common carp may be digested. Latterly, Jauncey (1982) in a review of carp nutrition has suggested that cellulase is not present in the gut of Cyprinus carpio. If cellulose is not utilised by carp, fibre in the diet merely acts as a bulking agent from which no energy may be taken, while energy is expended in its passage through the gut, and expulsion, a point made by Shiloh and Viola (1973) in work with carp substituting cattle manure for conventional feed materials. Its presence in the diet also takes the place of metabolisable carbohydrate which may be added to meet maintenance energy requirements. If there is a shortfall of dietary metabolisable energy in the form of carbohydrates and lipids, protein will be used for maintenance, rather than growth needs. Although amino acids are in some ways superior as a source of maintenance energy compared to carbohydrates (Jauncey, op. cit.), protein is an expensive dietary component, it is conventionally 'spared' by the inclusion of supplementary carbohydrates and lipids (Steffens,1981).

The low specific growth rates, and relative growth of the substituted diets may indicate that this phenomenon is taking place here: the protein being used for catabolic, rather than anabolic processes. The computed metabolisable energy, and measured fat levels in the diets would appear to be satisfactorily high, however.

Lack of access to the substituted protein source within the fish may also contribute to low utilisation levels: carp, although omnivores, have a relatively simple gut, and a short passage time of food through it (NAS,1977). The short time of exposure to active digestion processes may inhibit the utilisation of the protein which is intimately associated with paper.

5.5.3. Value of the Extract.

One problem in assessing the results is the departure of the actual, compared to the assessed protein contents of the diets. While the protein efficiency ratios were computed on the basis of the actual protein content, Ogino and Chen (1973) have demonstrated that the level of protein in the diet can affect both growth and protein utilisation in carp.

The variability in protein content of the paper based product has been discussed in Chapter 4, and this variability may have contributed to the low performance of the substituted diets. The standard errors of the mean values of protein content for these diets suggest that the protein contents may not have been uniform from day to day. It is probably not reasonable to consider the paper based extract as a standardised feed material. However, the intention of the feeding trial was to establish the feed value of the material, and this variability is an intrinsic characteristic. Within this framework, the substitute protein source has not displayed a high value. As the material did not generate equal growth or protein efficiency ratio performances compared to the main dietary compnent which it substituted, it must be ascribed a feed value (and consequently an economic value) of lower than that of wheat middlings. This is the case for carp, at least. Nehring (1965) has shown that the protein digestibilities of a variety of plant feed materials are greater for pigs than carp. This probably does little, however, to improve the value of this material.

6.AERATION 2.

6.1.Introduction.

The first attempt at separating the solid and liquid fractions of the effluent displayed two fundamental limitations. A direct interface between the liquid and the air allowed high evaporation, and ammonium nitrogen volatilisation. This led to a rapid loss of the aqueous phase and the nitrogen in it. A further problem lay in variability of solid attachment to the paper base, which gave serious restrictions on the value of the material as a substitute protein source in formulated feeds.

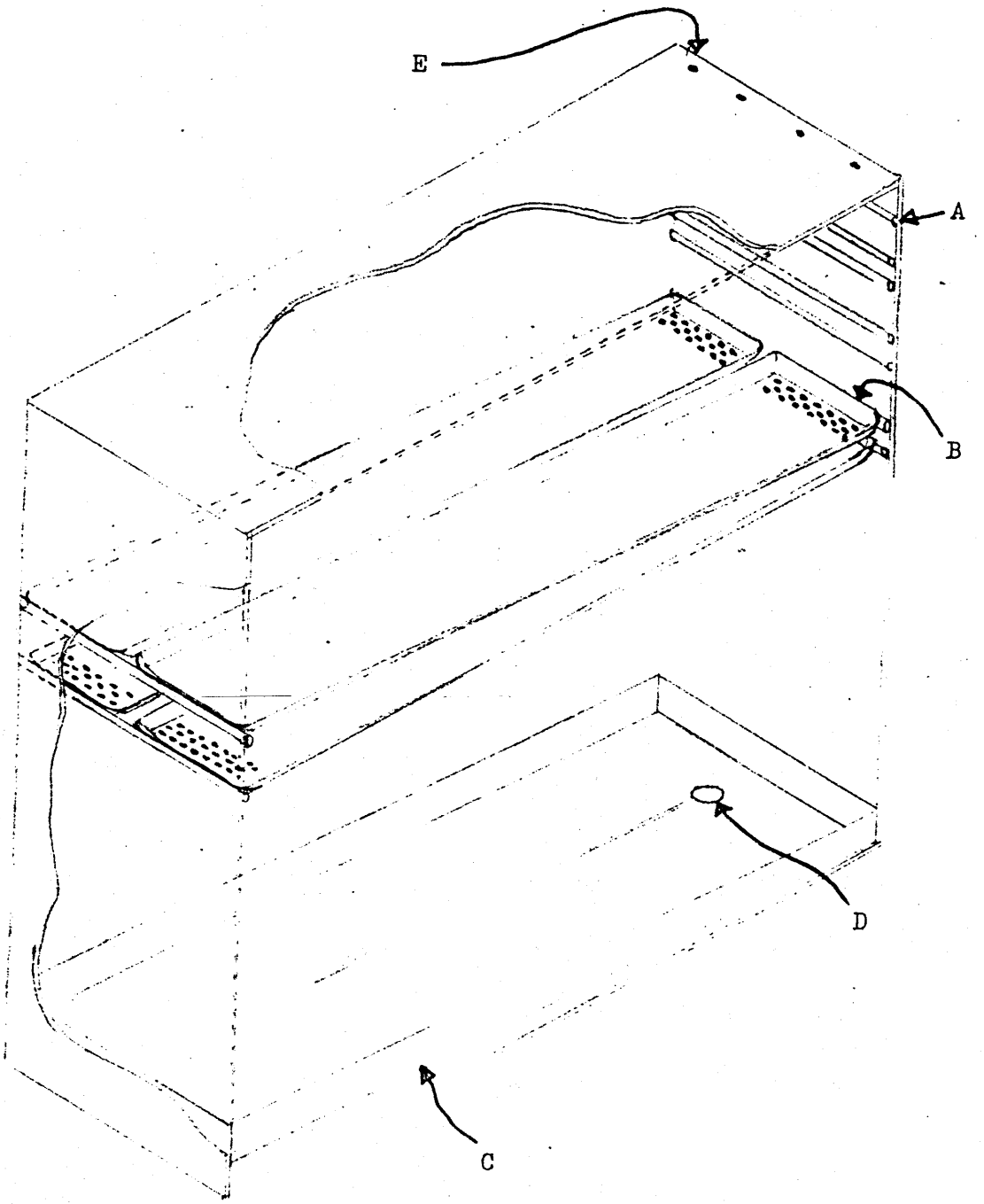
This chapter reports on modifications to the method of aeration, which are intended to reduce nitrogen losses from the aqueous phase, and give a solid with less variability. This was attempted by reducing access of the liquid to the air, and dispensing with the paper base.

6.2.Materials and Methods.

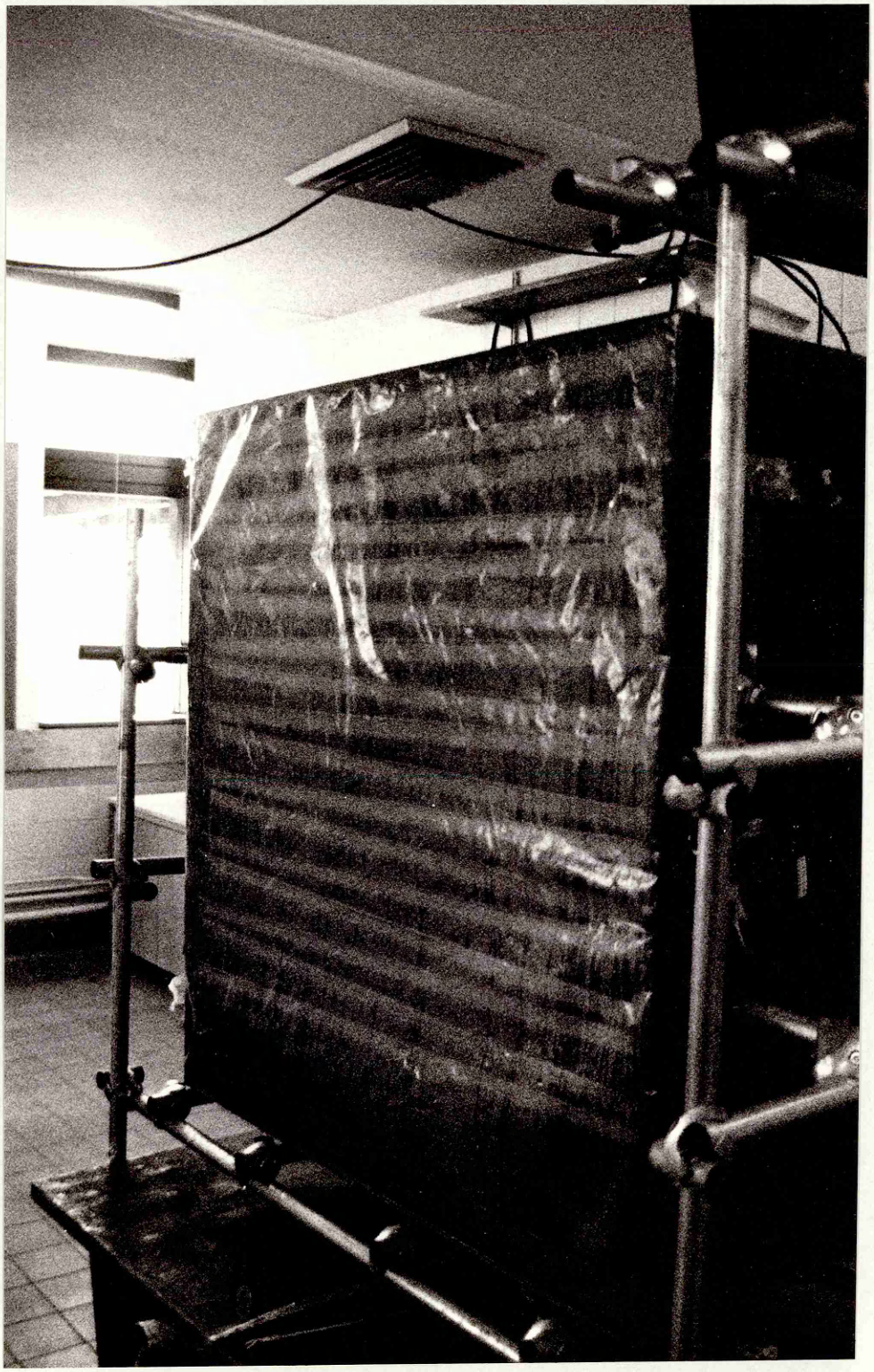
6.2.1. The Aeration Rig.

Figure 6.2.a. shows the rig used in these aeration experiments. This comprises a box with the external dimensions of 1.14M tall, 0.43M deep and 1.19M wide. The box was constructed of 10mm marine ply, and had no front. On the inside face of each of the end walls a series of 15 wooden battens (A) 20X5X420mm were secured horizontally. Each batten on one face was "paired" with a similar batten on the face at the opposite end of the box. The vertical drop from the top of one batten, to the top of its pair was 25mm. Each pair of battens formed runners, on which 2 plastic trays placed side by side (B) rested. The trays were 117mm long.Due to the battens being offset, a fall of approximately 1 in 50 was experienced at the surface of

Diagram 6.2.a. Cutaway Drawing Showing the Experimental Rig used for the Second Aeration Experiment. Not to Scale. Sump and Header Tanks not shown.



Photograph 6.2.a. Showing the filter with the polythene front cover *in situ*.



each tray. The pair of battens immediately below were offset in the opposite direction. The plastic trays were window box moisture retainers, 1.17M long, and .21M wide, the edges of which rose such that the flat bottom of the tray was a surface of 1.13M X.17M, surrounded by rising sides. On the outer edge of the rising sides was a 5mm lip. The flat lip on each end rested on the battens. At one end of each tray 20 holes, 5mm wide were drilled, affording drainage.

Into the base of the box was fitted a larger tray (C), 1.17 X.42M, with a vertical surround of 50mm, which was secured by means of glass reinforced plastic strips. This tray also was also fixed to give a drop of 1 in 50. At the lower end of this tray a drain hole (D), 30mm in diameter was drilled. A .3M length of 30mm plastic tubing was inserted in this hole, and led to a 250 litre capacity sump tank. The plastic trays were sandpapered, to give a key for biofilm development. The box itself was varnished. During each experiment the front of the box was covered by a heavy duty polythene sheet stretched taut, and tacked to the outside of the box. The bottom end of the polythene folded over the inside of the large tray C, so that condensation on the plastic would ultimately return to the sump.

The sump tank was covered with a sheet of 3 ply wood, into which 3 holes 50mm, 50mm and 10mm, respectively were drilled, to allow outlet and return flow pipes, aerator hose, and electric cable into the sump. A 50W submersible pump placed in the sump tank carried slurry to a 45 litre header tank secured above the aeration box. Slurry flowed via four 4mm internal diameter tubes, through 5mm holes drilled in the top of the box (E), at the rate of 500cm^3 per minute per set of trays. The slurry was allowed to flow down the inclined trays, to drain into the sump. Air was introduced into the slurry in the sump via a compressor and air diffusers, at the rate of 4 litres per minute. The completed filter with polythene cover is depicted in photograph 6.2.a.

6.2.2. Experimental.

Approximately 200 litres of anaerobically digested slurry from Botany Bay Farm was allowed to stand for 2 days, and a measured volume, between 100 and 150 litres, of the supernatant liquid fraction with solids less

than 1mm in diameter introduced into the sump tank, and the aerators and pump switched on.

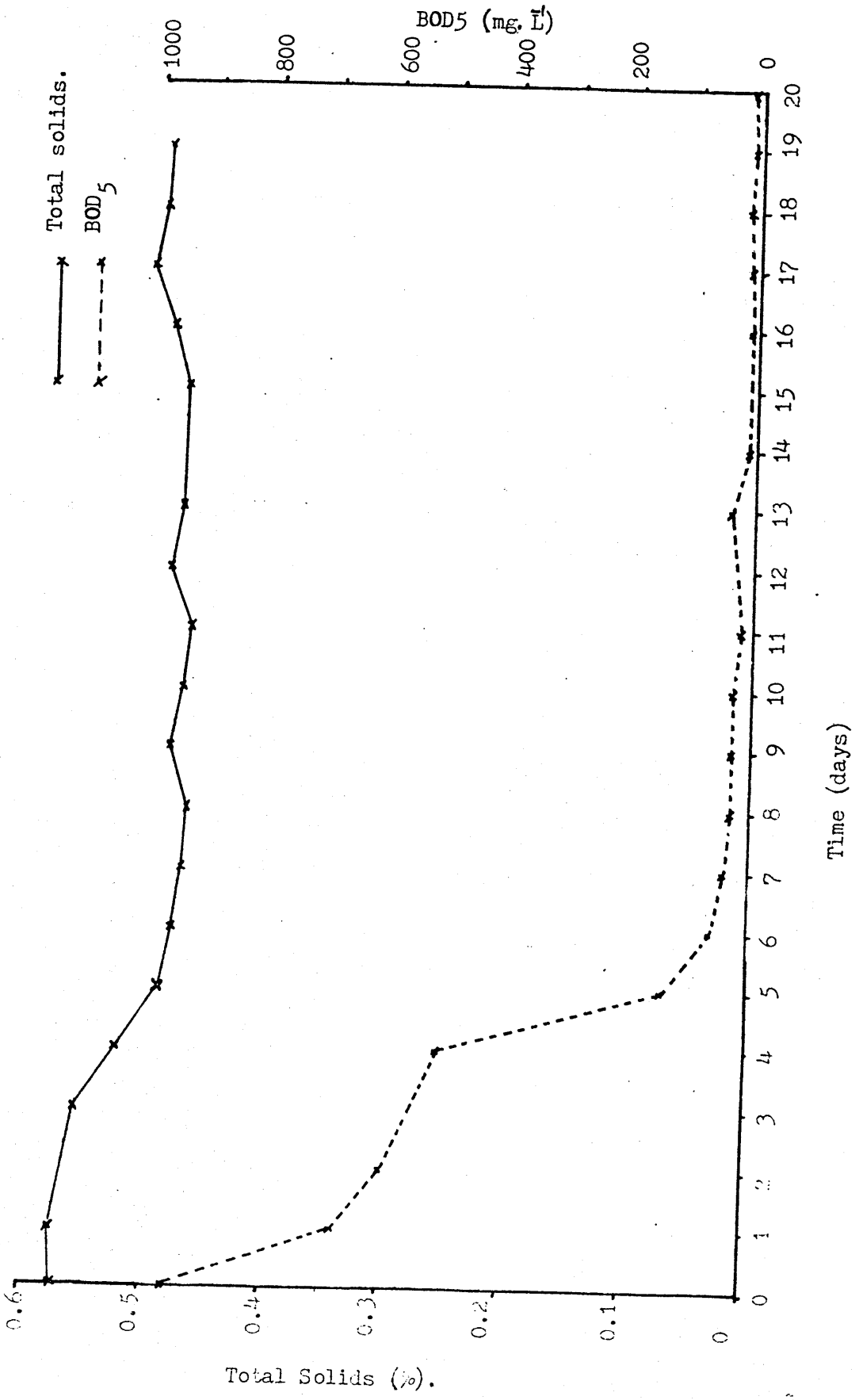
Samples from the bulk effluent in the sump were taken daily, and BOD_5, total solids, volatile solids, total nitrogen, ammonium nitrogen, and nitrate nitrogen were determined. Ammonium N was determined by the EIL ammonia ion specific electrode (EIL, Chertsey, Surrey.), BOD_5 by the standard wastewater analysis method (Anon,1971), total reduced nitrogen by a modified macro Kjeldahl method, total solids by the gravimetric method, and volatile solids by ashing the oven dried samples. Initial attempts to determine nitrate nitrogen were by the ion specific EIL nitrate electrode. As this electrode is very sensitive to interference by organic anions,however, this method was rapidly discontinued, and nitrate nitrogen was established by the semi quantitative Merckoquant test kit (Merck, Darmstadt, Germany), using amidosulphonic acid to eliminate nitrite interference. Nitrite nitrogen was determined by the semi quantitative Visocolor test kit (Macherey-Negel, Deuren, Germany).

Four experiments, lasting 20 days, were carried out using this rig. Table 6.3.2.a. gives details of the treatments. At the end of each experiment the volume of liquid remaining in the sump tank was established.When cleaned, the trays were scraped using a conventional rubber window wiper. The collected solids were air dried at 35°C, and the liquid placed in a holding tank. Samples of both the liquid and the solid were sent to ADAS for plant nutrient, and amino acid analysis respectively. For the latter two experiments a balance of nitrogen for slurry before and after aeration was carried out.

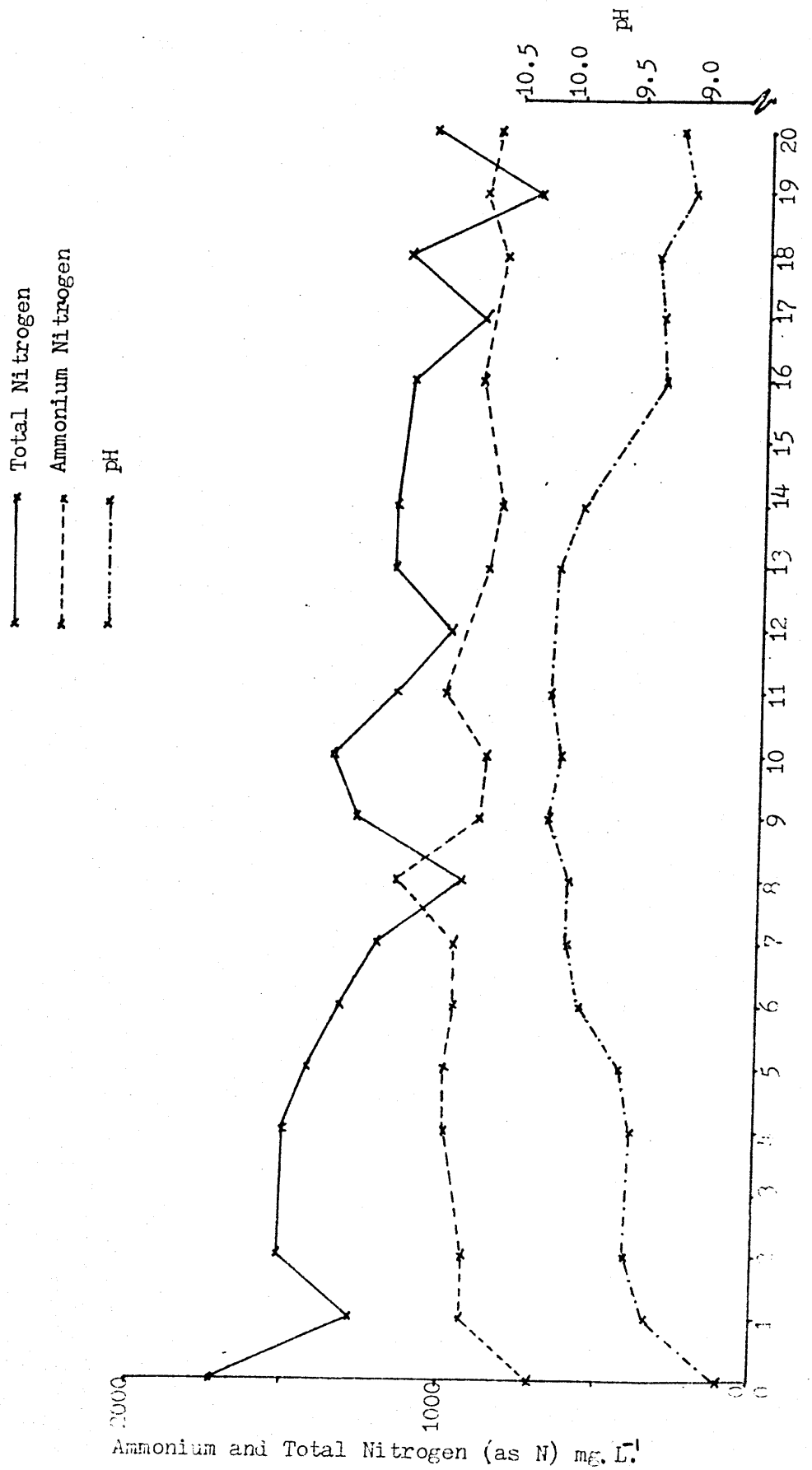
6.3.Results.

Graphs 6.3.1. a,c,e and g show the changes in solids content and decline of BOD_5 of the effluent for the four experiments, while graphs 6.3.1.b,d,f and h show changes in ammonium nitrogen, total nitrogen and pH for the same experiments, respectively. The first two experiments were carried out on effluent removed from the lagoon which receives slurry from the digester, while the second two were carried out on slurry taken directly from the

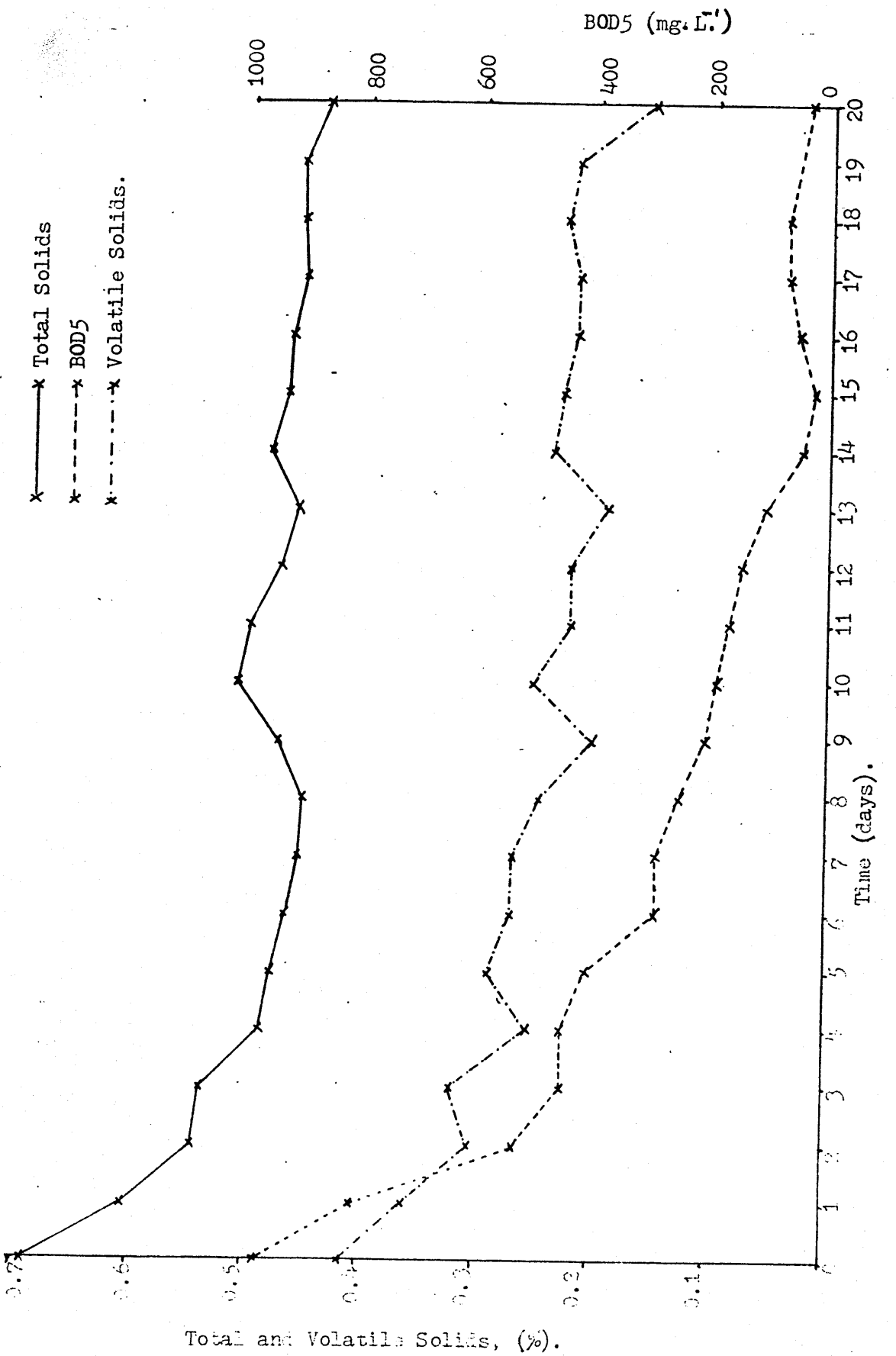
Graph 6.3.1.a. Change in BOD_5 and total solid content of slurry over time. Run 1.



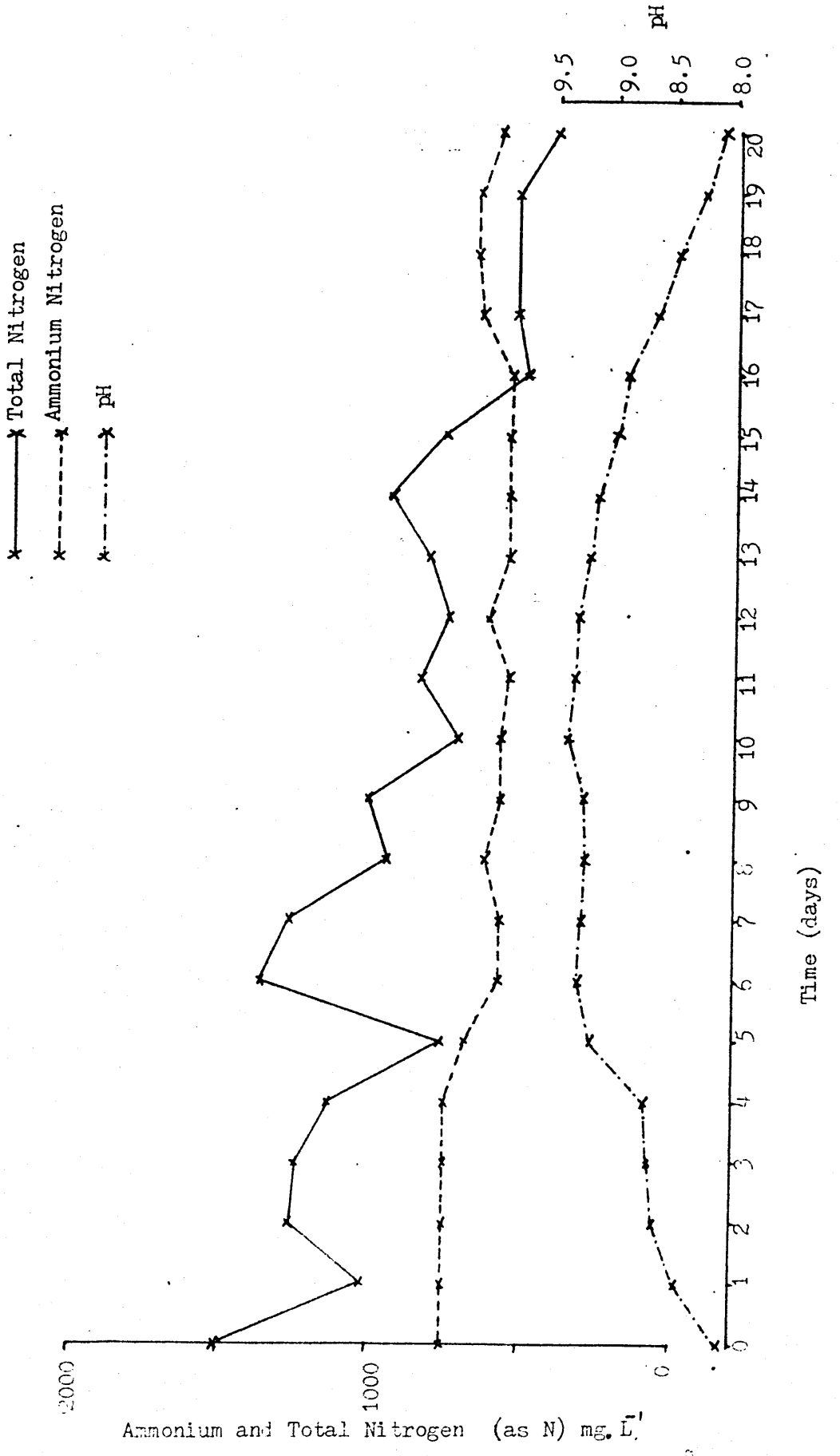
Graph 6.3.1.b. Change in Total Nitrogen, Ammonium Nitrogen, and pH of slurry over time. Run 1.



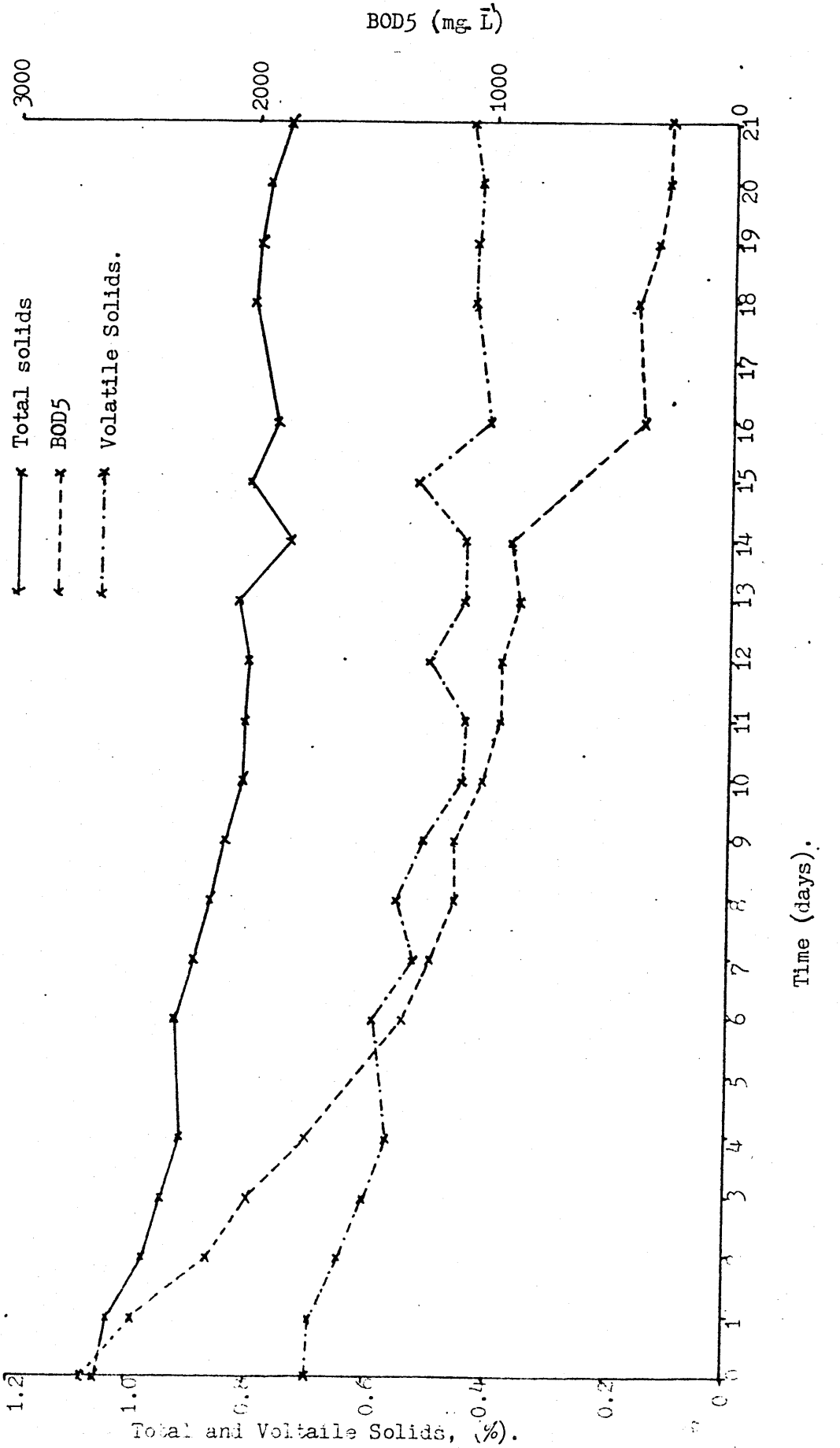
Graph 6.3.1.c. Change in BOD_5, Total solid, and volatile solid content of slurry, over time. Run 2.



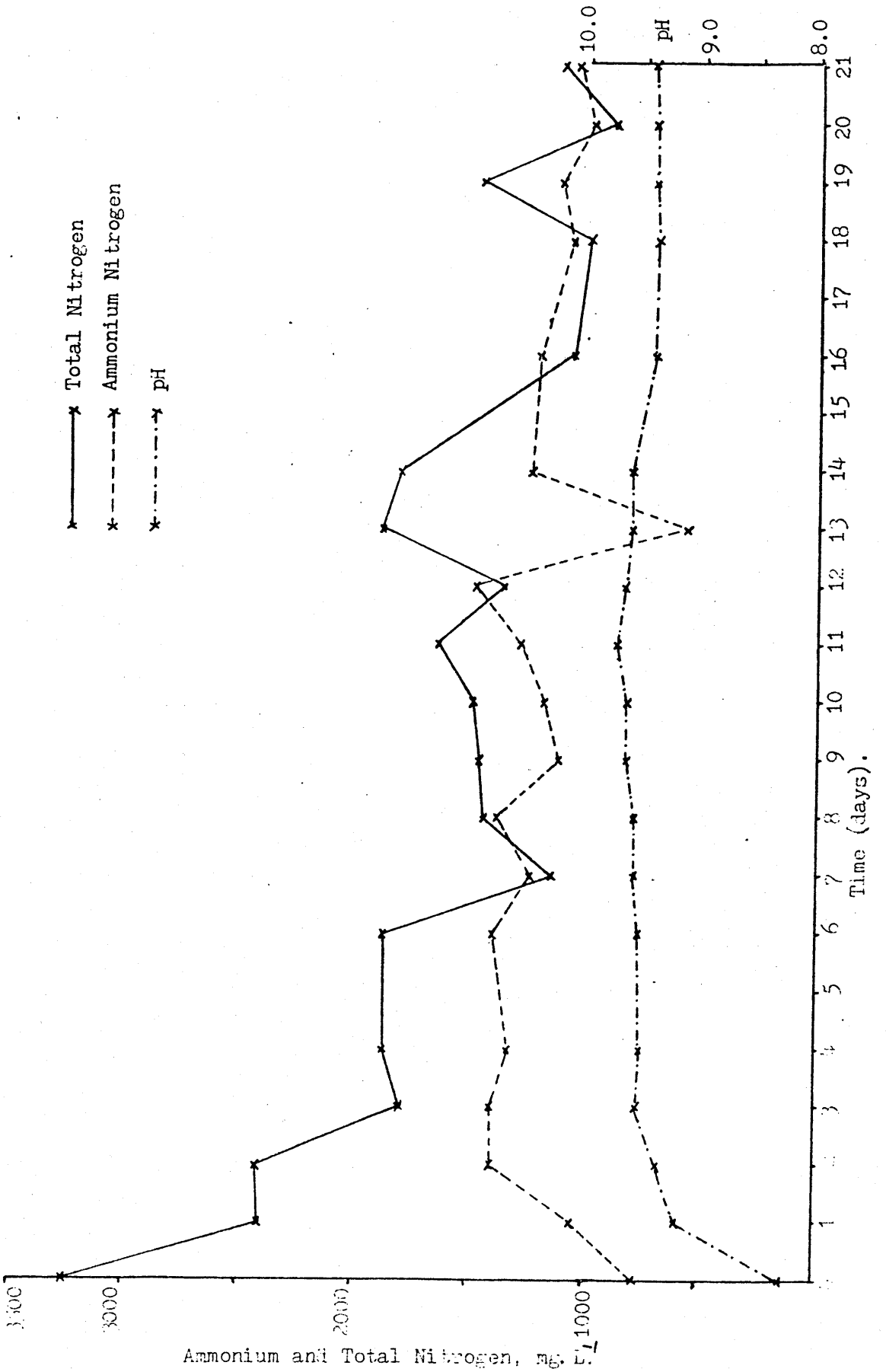
Graph 6.3.1.d. Change in Total Nitrogen, Ammonium Nitrogen, and pH of slurry over time. Run 2.



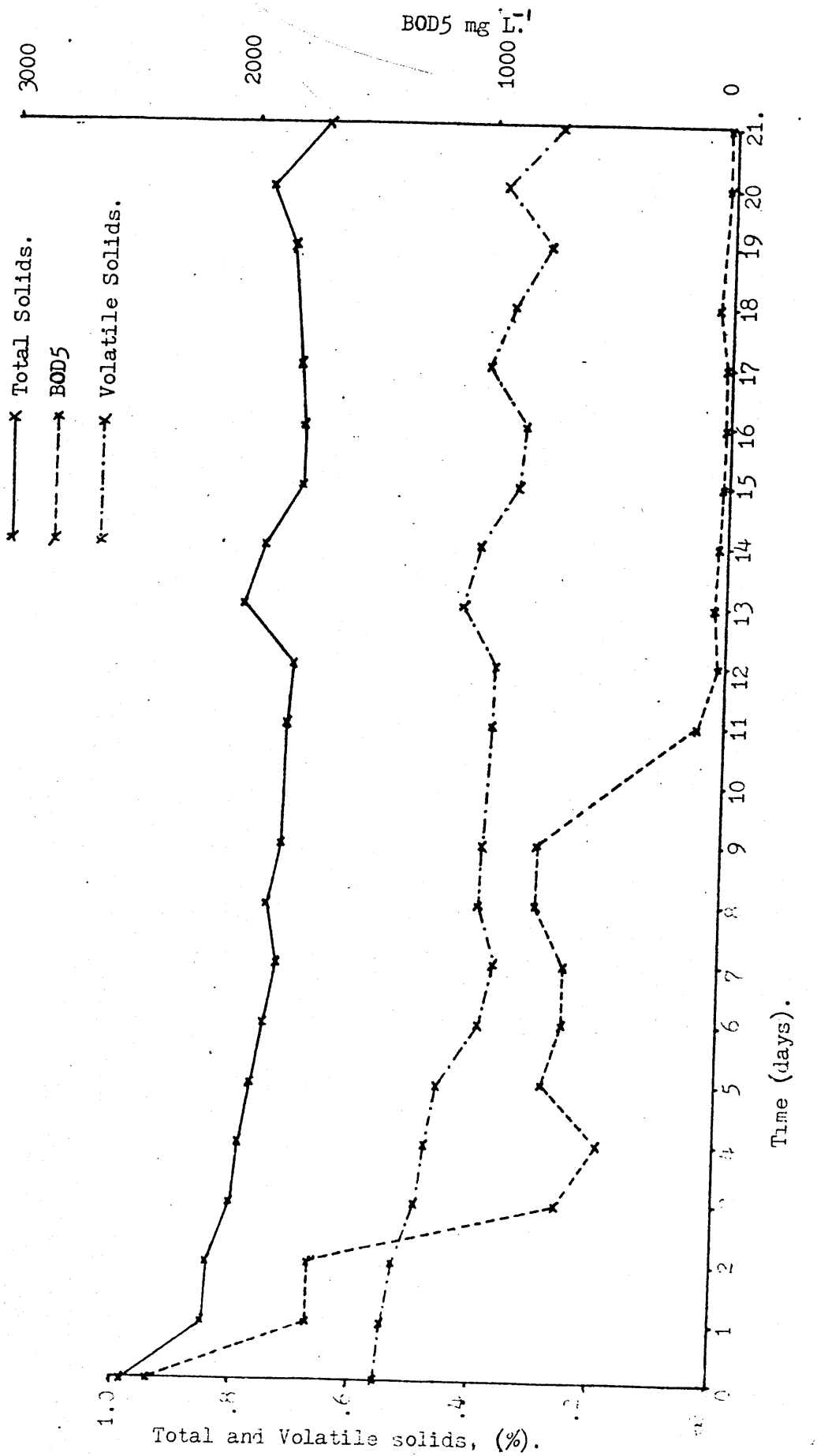
Graph 6.3.1.e. Change in BOD_5, Total Solid and Volatile Solid content of slurry, over time. Run 3.



Graph 6.3.1.f. Changes in Total Nitrogen, Ammonium Nitrogen, and pH of slurry, over time. Run 3.



Graph 6.3.1.g. Changes in BOD_5, Total Solid and Volatile Solid content of slurry, over time, Run 4.



Graph 6.3.1.h. Changes in Total Nitrogen, Ammonium Nitrogen, and pH of slurry, over time. Run 4.

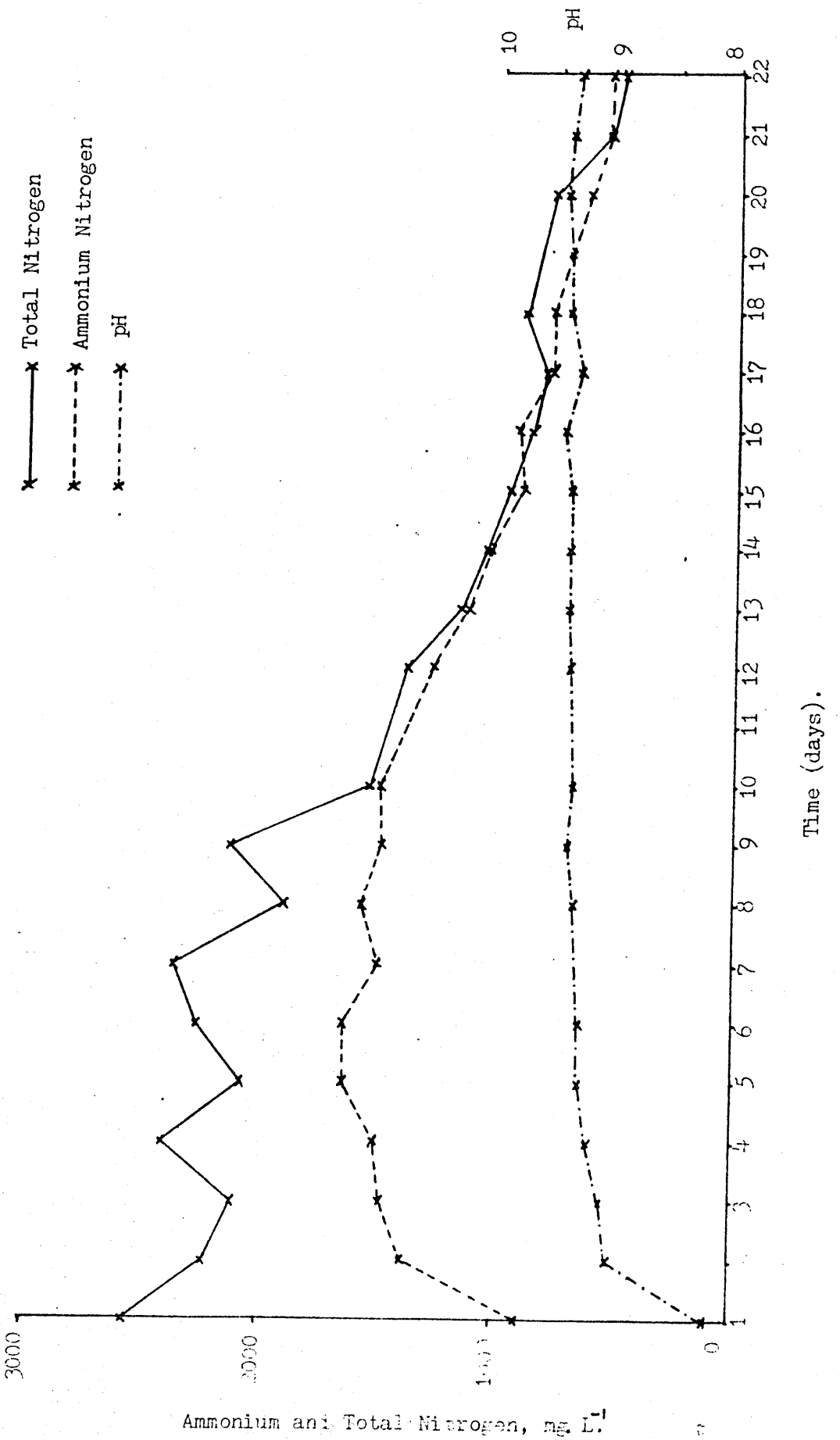


Table 6.3.1.j. Nitrogen in Slurry, and Removed, in Aeration Runs 3 & 4.

RUN 3.

	Initial	Final	Evaporative Losses (litres)
Volume of Liquid in the sump(L)	126.7	89	37.7
Kjeldahl Nitrogen ($mg.L^{-1}$ as N)	3259	1109	
Ammonium Nitrogen, ($mg.L^{-1}$ as N)	776	1049	
Total N in sump(g)	412.9	98.7	
Dry Weight of Solid Removed(g)		356	
Weight of N in Solids Removed (g)		22.25	
Total N	412.9	121.0	

RUN 4.

Volume of Liquid in the sump (L)	147	102	45
Kjeldahl Nitrogen ($mg.L^{-1}$ as N)	2565	475	
Ammonium Nitrogen ($mg.L^{-1}$ as N)	883	538	
Total N in sump(g)	377.1	48.5	
Dry weight of Solid Removed(g)		495	
Weight of N in Solids Removed(g)		24.80	
Total N	377.1	73.3	

Percentage of Nitrogen Accounted for:

Run 3	29.29
Run 4	19.44

Table 6.3.1.k. Nutrient and Sundry Water Quality Measurements, Aerated Effluent, Supernatant Fraction, Runs 1 and 2 mixed; and Runs 3 and 4.

	Runs 1 and 2	Runs 3 and 4
Nitrogen*	570	
Potassium	1375	
Phosphate	77	
Calcium	44	
Magnesium	2	
Iron	6.9	
Manganese	0.3	
Copper	4.4	
Molybdenum	6.4	
Zinc	3.4	
Sulphate	51	
Boron	1.2	
Sodium	60	
pH	8.80	9.35
Conductivity	6350mmhos. cm^{-2}	7843mmhos. cm^{-2}
BOD_5	30mg. L^{-1}	102mg. L^{-1}
COD	3847mg. L^{-1}	4487mg. L^{-1}
Total Solids	5700mg. L^{-1}	8742mg. L^{-1}
Suspended Solids	521mg. L^{-1}	784mg. L^{-1}
Volatile Solids	2287mg. L^{-1}	3053mg. L^{-1}

* All values as ppm unless otherwise stated.

Table 6.3.1.1. Proximate, and Amino Acid Analysis of the Solids Removed from the Aeration Box (Admixture of all runs).

	(%, dry weight basis).
Crude Protein	31.32(1.03)[a]
Ash	28.95(0.26)
Fat	0.76(0.01)
Fibre	<1.0[b]
NFE	~38.0

Air Dried at 35°C, Moisture Content 9.50(0.034)

Gross Energy Value 3452(70) kcal. kg^{-1} (14.44 MJ. kg^{-1})

Amino Acid Content g. $16gN^{-1}$.

Alanine	5.27	Methionine	1.73
Arginine	3.83	Phenylanaline	5.01
Aspartine	8.20	Proline	3.23
Cystine	1.85	Serine	3.87
Glucine	8.71	Threonine	4.20
Glycine	4.99	Tryptophan	c
Histidine	1.66	Tyrosine	3.00
Iso-Leucine	4.92	Valine	4.59
Leucine	7.39		
Lysine	3.74		

a All values in brackets are standard errors of the mean.

b Derived Value.

c Destroyed by acid hydrolysis.

digester. Due to dilution with rainwater and, presumably, some surface aeration, total and ammonium nitrogen, biochemical oxygen demand and total solids are lower in the effluent from the lagoon.

One of the benefits of anaerobic digestion is the large reduction in BOD_5. The removal of the remaining oxygen demand can be rapidly accomplished by this single stage aeration process. Aeration also achieves appreciable reductions in solids content, but the graphs do not indicate a high correlation between solids removed and BOD_5 removal.

For all the experiments almost total removal of organic nitrogen was achieved during the 20 days. A component of this is ammonification (with a concomitant rise in pH) after the start of aeration. At no time during any of the experiments was nitrite or nitrate nitrogen detected. Table 6.3.1.j. gives a balance of nitrogen, and volume of effluent before and after the aeration process for the latter two runs. Nitrogen losses are relatively small, compared to conventional aeration techniques.

Tables 6.3.1.k. shows the plant essential nutrient profile of the final effluent (combined) from the first two runs, together with total solid content, final BOD_5, COD and electrical conductivity, together with a limited profile of the liquid from the latter two runs. Table 6.3.1.l. lists the amino acid content (g.A.A. $16\bar{g}^{1}N$) for the collected solid harvested from the trays (an admixture from all experiments), together with crude protein, fat and ash contents.

6.4.Discussion.

6.4.1.General.

This somewhat unconventional aeration design owes something to both the previous aeration system-the trickling filter-and rotating biological contactor (RBC) design. The RBC comprises a series of discs aligned about a single axis. Approximately half the area of each disc is immersed in the effluent wastewater. The discs rotate, so that the period of immersion is followed by a period of contact with the air. The fixed biofilm grows on

the discs. Oxygen is taken into (and CO_2 removed from) the liquid film covering the biofilm. Re-immersion of the biofilm in the effluent brings the microbial population into contact with the substrate. Some (American) systems employ enhanced oxygen atmospheres above the discs, and/or enforced aeration in the bulk liquid, to aid the process (Grady and Lim,1981).

For the aeration system employed here the biofilm develops on the planar surfaces. The effluent comes in contact with the biofilm by trickling down the trays. Due to the need to restrict evaporation and volatilisation losses, oxygen is provided not by contact with the air, but by enforced aeration of the bulk liquid. The level of aeration is given by the need to keep the liquid aerobic. Classically, the level of dissolved oxygen below which the bacterial population will become inhibited (i.e. the bacterial transformations will be oxygen concentration dependent) varies from 1.5mg. L^{-1} to 2.5mg. L^{-1} depending on author (Loehr,1974).As the atmosphere within the box is effectively anaerobic, the simplest means of ensuring sufficient oxygen delivery to the system is by maintaining the O_2 concentration of the liquid leaving the box above 2.5mg. L^{-1}

6.4.2.Nitrogen Transformations.

One of the aims of the aeration process was to bring about the transformation of ammonium nitrogen to nitrate nitrogen. As neither nitrate, nor the intermediate product nitrite were detected at any point, and ammonium concentration remained high,and was the predominant species at the end of each run, this objective has not been met.

The main constraints to nitrification in aeration systems are biochemical oxygen demand, which must be below 50mg. L^{-1} and oxygen levels, which must be above 2.5mg. L^{-1} For the first and fourth experiments, the critical BOD_5 level was quite rapidly reached, and rather more slowly in the second run. Nitrification has been readily achieved by other workers when aerating pig effluent, starting from much higher BOD levels (Osborne et al.,1976; Hepherd and Charlock,1971). The key difference in the present case appears to be pH: Eckenfelder (op.cit.) in a review of conditions required for the microbial transformation of nitrogen, indicates a very

rapid falling off in activity of Nitrosomonas spp. at pH values above 8. At the pH's encountered in this work, due to the initially high values and rises due to ammonification, activity is negligable. Nitrobacter spp. are slower growing and are more susceptible to high pH than Nitrosomonas. Murray, Parsons and Robinson (1975) demonstrated nitrite accumulations in aeration work with farm animal wastes with high levels of ammonia. Between the first and second experiments the biofilm was left in situ to allow for possible slow growing of these bacteria, but no difference in nitrogen status was observed.

High levels of nitrogen loss, due mainly to the mechanism of ammonia volatilisation, are a common feature of aeration systems. Some, like those of Vanstaen et al.(1976) and Loynachan et al.(1976) are designed with this feature specifically in mind. As pH reductions are due (initially) to ammonia volatilisation, and then to the production of protons by nitrification (Owens et al.1973), a system which inhibits nitrogen loss in this way is unlikely to achieve a sufficiently low pH for bacterial nitrification. One way of producing conditions better suited to nitrification would be to add phosphoric acid at the outset. Bartlett et al(1978) found that adding phosphoric acid to anaerobically digested dairy manure reduced the rise in pH associated with subsequent aeration. Rises in pH did occur, however, probably due to the buffering ability of digested slurry, noted in **Appendix III.**

6.4.3. pH.

Apart from the problems of the inhibition of nitrification, high pH causes difficulties if the resultant liquid is to be used for hydroponics. Garraway (1981) has pointed out that considerable increases in soluble phosphate and magnesium accrue when pH is reduced as a function of nitrification. Calcium can be added to these two. Trace elements, notably iron, manganese and boron may also be precipitated at high pH's (Russell, 1973).

6.4.4. Solids Removal.

The graphs of changes in measured parameters show that high levels of solid removal from the bulk effluent were not achieved. This indicates that the solids removed from the system by scraping the plastic trays did not constitute a large proportion of the total solid in the effluent, the opposite of what was anticipated. Furthermore, the constant agitation of the bulk effluent would maintain the circulation of any solids in the sump tank, which otherwise would be settleable. The final effluent (as shown in Table 6.3.1.k.) indicates an appreciably lower solids content than the levels in the sump for the first two runs, a point borne out by the low nitrogen recovery levels for the latter two runs (Table 6.3.1.j.). At the end of each run the aerators and pump were switched off, and the effluent allowed to settle for a day. After this period the effluent was decanted and removed. An appreciable sludge was noted in the bottom of the sump after the supernatant had been decanted. In addition, at the end of the experimental cycle when the rig was dismantled, the header tank feeding the aeration box was seen to be full of sludge. This tank had been acting as a settlement tank over the experimental period. The solids lost in this sludge, and those that had accumulated in the bottom of the sump tank after final settlement, go some way to explain the low N recovery values. Most of the nitrogen recovered was in the form of ammonium N. These factors are a clear indication of the lack of success of the aeration box as a means for removing the suspended solids: it would appear that the aeration process resembles an activated sludge sytem, rather than an RBC. Under these circumstances an (enclosed) final settlement tank with daily loading of the system would result in improved N recovery. Further development work on an aeration system designed to produce materials for further food production operations should pursue this line. In this work, however, the engineering inputs were largely empirical, and not geared to optimisation.

6.4.5. Resultant Materials.

The nutrient profile of the liquid from the first two runs is shown in table 6.3.1.k. Although the liquid is relatively high in zinc and copper, and low in phosphate, calcium and magnesium, the departures from the optima descibed for liquids for use in NFT do not appear too great. The BOD_5 has

been substantially reduced, and the oxidation process, Garraway and Ramirez'(1982) work suggests, will have substantially reduced the phenolic acid content of the liquid. Titration of the supernatant liquid with phosphoric acid revealed no residual buffering capacity. Whether the high ammonium nitrogen content remaining in the system would present difficulties in the use of the liquid for hydroponics, can only be established by in vivo investigation.

Table 6.3.1.1. shows the analysis of the solid material collected from all runs. Although the ash content is very high, and the fat content low, the crude protein is sufficiently high to enable the material to be used as a replacement feed material. The amino acid profile is also presented: although the cystine and (more importantly) lysine levels are quite low, the methionine level, frequently the first limiting essential amino acid in feed formulation, is high.

7.PROTEIN EXTRACT AS A SUBSTITUTE FEED MATERIAL FOR FISH.

7.1. Introduction.

The chapter reports on an experiment designed to quantify the feed value of the microbial protein extract, generated by the work reported in chapter 6. The material is tested on common carp.

7.2. Materials and Methods.

7.2.1. Experimental.

The experimental rig used in the previous feeding trial was used for this work, with some minor modifications. The shower water heater unit was replaced with a 3Kw kettle element secured in the side of the header tank. A baffle was placed around this element such that incoming water had to pass over the kettle element before passing into the main bulk of the tank. The kettle element was thermostatically controlled, and set at 24°C. In each aquarium a 200 watt thermostatically controlled submersible aquarium heater was placed, set at 24°C. In this way water temperature was maintained at 24±0.5°C at all times. Incoming water was filtered by a commercial activated carbon water filter (Fileder Systems, Wateringbury, Kent). Pellet size was given by those solids which were smaller than 3.15mm, and larger than 2.0mm. The feed materials were dried at 30°C in a fan convector oven. Faecal samples were collected in the final 20 days of the experiment, and apparent digestiblity was established by a modification of the method of Furukawa and Tsukahara (1966). The experiment lasted 74 days. Fingerling mirror carp, of 4.5g average weight, were obtained from Cotswold Carp Farm, and randomly allocated to the 12 tanks, 11 fish per tank, and allowed to equilibrate. At the start of the experiment one fish from each tank was removed, sacrificed, oven dried

Table 7.2.a. Dietary Components for the Three Experimental Diets. (%, dry weight basis.)

Component	Control	10% Subst.	20%Subst.
Fish Meal	35	30	30
Soyabean Meal	21	23	16
Extract	0	10	20
∝Cellulose	22.5	15.5	12.5
Starch	10	10	10
Cod Liver Oil	3	3	3
Corn Oil	2	2	2
Binder[a]	3	3	3
Vitamin Mix[b]	2	2	2
Mineral Mix[b]	1	1	1
Chromic Oxide	0.5	0.5	0.5

a Carboxymethylcellulose, disodium salt.

b Vitamin and Mineral Mixes were supplied by B.P.Nutrition U.K., Ltd., and are formulated to satisfy these requirements for carp.

Table 7.2.b. Amino Acid Contents of the Dietary Components.
(g A.A. 100 g^{-1} dry feed material.)

Amino Acid	Fish Meal	Soyabean Meal	Extract
Arginine	5.17	3.28	1.33
Histidine	1.84	1.22	0.57
Leucine	5.69	3.99	2.55
Iso-Leucine	3.43	3.11	1.70
Lysine	6.30	3.05	1.30
Methionine	3.14	2.01	0.60
Cystine	0.82	0.57	0.64
Phenylanaline	2.99	2.34	1.73
Tyrosine	2.43	1.55	1.04
Threonine	3.15	1.89	1.45
Tryptophan	0.85	0.69	-
Valine	4.78	2.45	1.59
International Feed Number	5-02-000	5-04-600	

Table 7.2.c. Essential Amino Acid Contents of the Diets (g A.A. 100g⁻¹ of dry feed), compared to the Requirements of Carp.

Amino Acid	Control	10% Substitution	20% Substitution	Requirements
Arginine	2.50	2.44	2.34	1.7
Histidine	1.59	0.89	0.86	0.8
Leucine	2.83	2.88	2.86	1.3
Iso-Leucine	1.85	1.91	2.87	1.0
Lysine	2.85	2.72	2.64	2.2
Methionine	1.52	1.46	1.38	1.2 or 0.8
Cystine	0.41	0.44	0.47	0 or 2.0
Phenylanaline	1.54	1.61	1.62	2.5 or 1.3
Tyrosine	1.18	1.19	1.19	0 or 1.0
Threonine	1.50	1.53	1.54	1.5
Tryptophan	0.44	0.41	0.37	0.3
Valine	2.19	2.16	2.14	1.4

Table 7.2.d. Proximate Analysis of the three experimental diets.

Component	0% Subst.	10% Subst.	20% Subst.
Protein (%)	34.51(0.22)	36.31(0.31)	37.79(0.73)
Fat (%)	7.50(0.03)	7.27(0.01)	4.32(0.02)
Ash (%)	7.08(0.07)	9.62(0.07)	12.15(0.11)
Gross Energy ($MJ.Kg^{-1}$)	20.15(0.27)	19.32(0.18)	18.33(0.10)

Table 7.2.e. Estimated Metabolisable Energy in the Diets. ($kcal.kg^{-1}$.)

	0% Subst.	10% Subst.	20% Subst.
Fish Meal	1244	1067	1067
Soyabean Meal	568	622	433
Extract	-	200	400
Starch	325	325	325
Cod Liver Oil	248	248	248
Corn Oil	198	198	198
Total ($kcal.kg^{-1}$)	2583	2660	2671
Total ($MJ.kg^{-1}$)	10.82	11.14	11.19

and ground. The resultant grindings were pooled and subsamples taken for the determination of apparent net protein utilisation. In all other respects the experimental methods were the same as the previous work.

7.2.2. Feed Formulation.

Table 7.2.a. shows the percentage of dietary components in each of the three experimental diets. As the extract has a higher protein content than the paper based material, it may be used as a substitute for fish meal and soyabean meal. In the 10% diet, 5% of the fish meal is substituted and a small increase in soyabean meal is required. For the 20% substituted diet both fishmeal and soyabean meal requirements are lowered by 5%.

The essential amino acid requirements of carp are met by all the diets. Table 7.2.b. shows the essential amino acid contents of the dietary components. For this work the amino acid contents of the conventional feed materials were taken from the National Academy of Science(1977) tables. Table 2.7.c. is a summary of the essential amino acids delivered by each of the diets, together with the requirements for carp. The proximate analyses of the diets are shown in table 7.2.d.

Table 7.2.e. shows the metabolisable energy contents of the diets. The values for the conventional dietary sources are taken from NAS (op. cit.) tables, while an estimate of 2000 kcal/kg (8.38MJ/kg) is made for the extract.

7.3. Results.

7.3.1. Growth.

Graph 7.3.a. shows the mean relative growth for the three diets over the 74 day trial. Both the 10% and 20% diets outperformed the control diet, with the 10% diet having a slightly better performance than the 20%. This is reflected in the specific growth rates, shown in table 7.3.b. There is no significant difference between the substituted diets, but the fish on the 10% susbstituted diet had an overall specific growth rate significantly

Graph 7.3.a. Mean Relative Growth for the three treatments.
(Indexed from 100).

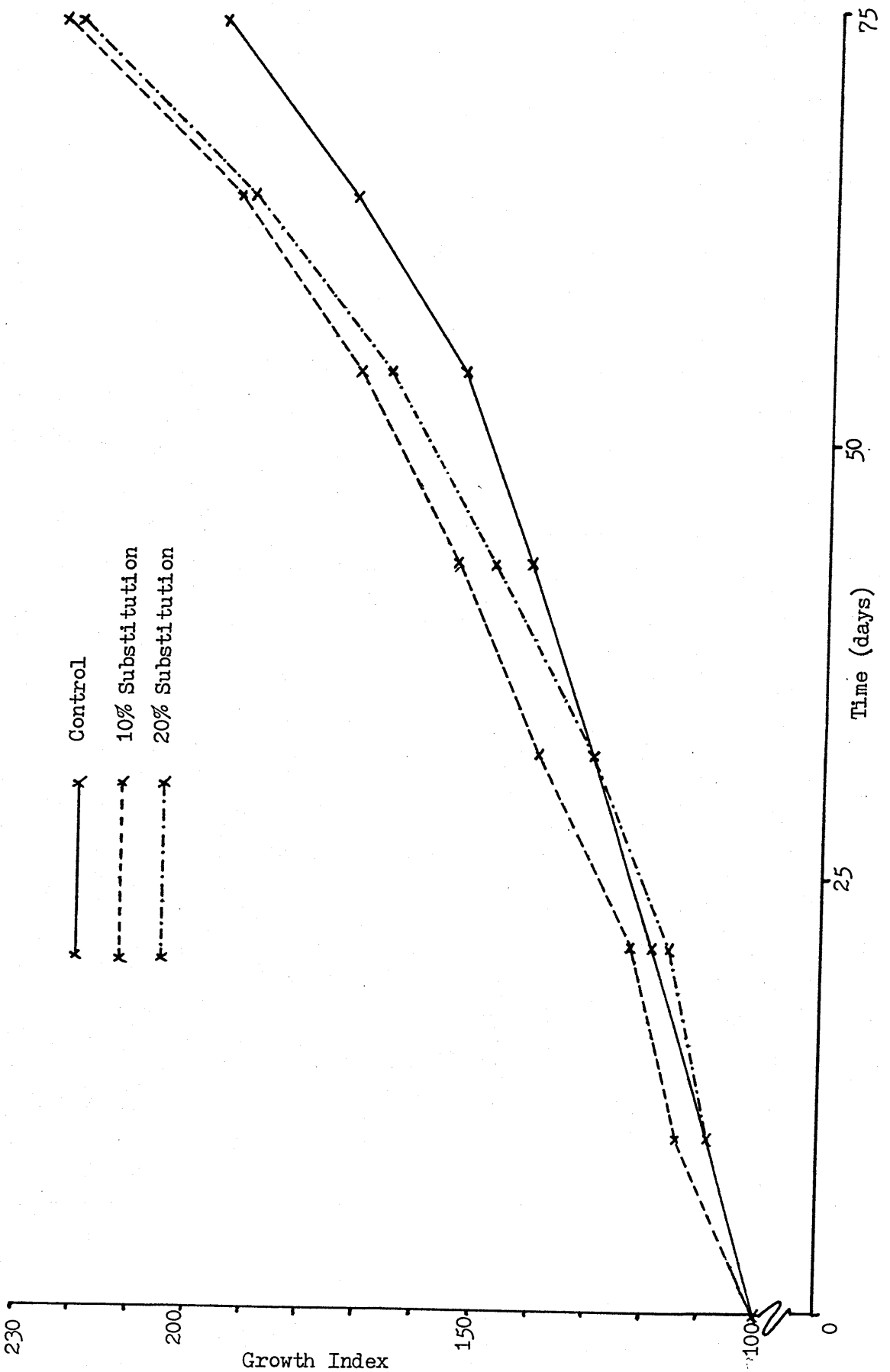


Table 7.3.b. Growth and Protein Utilisation Parameters, by treatment.

	Control	10%	20%
Feed Conversion Ratio.	4.22(0.25)^{a}	3.54(0.20)^{a}	3.50(0.22)^{a}
Specific Growth Rate.	0.91(0.03)^{a}	1.09(0.05)^{b}	1.07(0.07)^{ab}
Protein Efficiency Ratio.	0.69(0.04)^{a}	0.79(0.05)^{a}	0.77(0.05)^{a}
Apparent Net Protein Utilisation.	0.42(0.03)^{a}	0.52(0.03)^{a}	0.52(0.04)^{a}
Digestibility.	46.28(6.17)^{a}	39.34(2.89)^{a}	42.51(6.03)^{a}

Table 7.3.c. Analysis of the Culled Fish, All Treatments.

	Control	10%	20%
Protein(%)	55.63(1.41)^{a}	59.62(0.73)^{b}	59.80(1.07)^{b}
Fat(%)	30.82(1.24)^{a}	26.58(0.70)^{a}	19.78(1.71)^{b}
Ash(%)	9.20(0.19)^{a}	10.85(0.20)^{a}	13.39(0.70)^{b}

Superscript letters indicate homogenous subsets at the $P<0.05$ level.

higher than the control.

7.3.2. Nutrient and Protein Utilisation.

Table 7.3.b. also summarises the feed utilisation parameters for the three diets. There are no significant differences between either substituted treatment and the control for feed conversion and efficiency ratios, and apparent net protein utilisation and digestibility values. The feed conversion ratios are uniformly low. A value of approximately 2.5 is expected for carp at that water temperature (NAS, 1977).

7.3.3. Carcass Analysis.

Table 7.3.c. shows the mean fat, protein and ash contents of the fish culled at the end of the experiment. The fish on both substituted diets have a higher protein content than the control. The fish on the 20% diet have a significantly lower fat content, and higher ash content than those of both the control and 10% diets. These results directly reflect the proximate analyses of the diets themselves (Table 7.2.d.), insofar as the control diet has the lowest determined protein content, while the 20% diet has the lowest fat and ash contents.

7.4.Discussion.

While the feeds formulated for the first experiment were based on experimental diets used by Tacon and Ferns (1976) in work on the use of activated sludge in trout diets, this feed is based on diets used by Atack, Jauncey and Matty (1979), and Viola, Mokady and Arieli (1983) for nutritional experimentation on carp. It has fewer components, and has the advantage of being easily modified to these specific experimental needs. As the s.c.p. extract used in this trial has a higher protein content than the paper based material tested in Chapter 5, it may be used as a substitute for fish meal and soyabean meal.

The low fat level in the 20% diet is due to the replacement of both the high fat conventional feed materials with the low fat extract. Similarly, the ash content of the diets increases with substitution, producing a

significantly higher ash content in those fish. The high ash in the diet, and resultant raised ash in the fish, may have contributed to the lower growth of the 20%, compared to the 10% diet. Although this difference is not significant, it was persistent over the growth period. The 12.15% ash content of the 20% diet is as high as is desirable in the diet of fish (Stickney,1978).

The estimate of 2000kcal. kg^{-1} (8.38MJ. kg^{-1}) metabolisable energy for the extract is derived from the gross energy value of the material, in conjunction with the known m.e. values of other feed materials. The assumed percentage of total energy which is considered to be metabolisable is 58%, compared to 56% for soyabean meal, and 52% for yeast. This slightly high estimate is a round figure value which is a reasonable first approximation.

On the basis of the calculated value of the extract, the substituted diets have slightly higher energy values than the control diet. The variation is very small, however (only 3.4% difference betweeen the highest and lowest diets). Sin (1973) gives an m.e. optimum of 3000kcal. kg^{-1} (12.6MJ. kg^{-1}) of feed for carp. All three diets are suboptimal on this basis, but this is acceptable as the variation is so small. Such values are common in the literature.

One drawback in the low m.e. values of all diets is the diversion of energy from growth to maintenance energy needs. This may go some way to explain the uniformly low feed conversion ratios, although the low digestibility values indicate that other factors may have some influence. The role of water quality is discussed in **Appendix IV.**

The closeness in performance terms between the control and substituted treatments for the measured growth and nutrient utilisation parameters enables a straightforward assessment of the value of the protein extract. By assuming that no difference between the diets exists, then the value of the extract can be estimated at 43% that of fishmeal, or 70% that of soyabean meal. The low fat and high ash levels indicate that a ceiling for substitution may reasonably be set at 20%, in the diet of fish, at least.

8.OXIDISED PIG EFFLUENT AS A HYDROPONIC MEDIUM.

8.1. Introduction.

The criteria for successful separation are in part given by the in vitro chemical characteristics of the clarified liquid. More importantly, it is neccessary to establish the performance of the liquid in an NFT application. Previous hydroponic experimentation (reported in **Appendix 3**) demonstrated that the pig effluent has several major limitations to its use:- the presence of ammonium nitrogen, potentially giving rise to root death and blossom end rot, phenoxy compounds which may give rise to growth aberrations, the high BOD_5 of the liquid, which competes with the roots for dissolved oxygen in the solution itself, and the ratios between the nutrients, causing marked deficiencies. Other problems of lesser importance are the presence of suspended solids, whose surface activity render pH control difficult, and the potentially detrimental effect of sodium and its accumulation in the solution.

This early work also raised an interesting side issue in the debate on the suitability of effluent waters as a medium for hydroponic plant production: whether the measurement of electrical conductivity is a sufficiently good index of nutrient status in a solution whose nutrient ratios are such a departure from the theoretical optima, and which contains relatively high levels of non-essential electrolytes.

This experiment seeks primarily to establish whether the liquid clarified by the aeration process is suitable for use in NFT. In addition, the impact of sodium on yield, and the reliability of electrical conductivity as a nutrient status measurement, will be examined.

8.2.Materials and Methods.

8.2.1.The NFT System.

This experiment was carried out using the system employed in Appendix III, with certain modifications. Each of the 4 sump tanks serves a single "Layflat" channel of 12 metres, housed in an aluminium tray. Solution recycling is achieved with Nova 300W submersible, self priming pumps (Interdab Ltd., Leeds). Flow rate is controlled by a bipass system of 2 taps, permitting the solution to be pumped via 3cm tubing to the top of the run, or directly back into the sump. Float switches are fitted to each pump to prevent damage in the event of the sump emptying. Continuous monitoring of pH and conductivity levels is afforded by diverting some of the flow via 1cm tubing spliced into the main pipe to pH and Cf electrodes housed in flow cells linked to the appropriate meters (R.Crane Ltd.,Andover).The tomato plants are supported by baling wire strung from the tubular frame of the polytube. Each tank contains 100 litres of solution.

8.2.2.The Nutrient Solutions.

The four solutions employed were Libsol; Libsol with sodium; digested, oxidised pig effluent (DOPE) with potassium (hereafter DOPE-K); and DOPE with added nutrients (K-Rel). Table 8.2.2.a. shows the composition of the solutions. Libsol is a commercial NFT solution. The DOPE with Potassium comprises 30 litres of the effluent and 70 litres of water, giving a conductivity of 2.5mmhos. cm^{-2} Potassium in the form of K_2SO_4 is added to raise the N:K ratio from 1:2.644 to 1:3.202, the ratio in the Libsol solution. No other additions are made. The DOPE with added nutrients is formed by diluting the effluent with water until the potassium level is identical to that of Libsol (22.5 litres with 77.5 litres of water). This gives rise to a shortfall in other nutrients compared to Libsol. These are made up by the addition of inorganic salts. The fourth treatment comprises conventional Libsol with sufficient NaCl to raise the sodium level to that of the DOPE and nutrient treatment. Similar procedures are followed for the make-up solutions, employed when solution conductivity reaches 2 mmhos. cm^{-2} The DOPE with potassium make-up solution comprises 24.2 litres

Table 8.2.2.a. Nutrient Solution Compositions.

	Libsol Starter	Libsol Make-up	Libsol+Na Starter	Libsol+Na Make-up	DOPE +K Starter	DOPE+K Make-up	K-Rel Starter	Salt (weight g)	K-Rel Make-up.	Salt
N	104	49.3			156	117	126	-	53.9	-
P	62	0			23	17.3	24.4	H_3PO_4 (pH)	8.0	(pH)
K	333	142.6			500	375	333	-	142.6	-
Ca	168	68	As Libsol	As Libsol	13.2	9.9	10.7	$CaCO_3$(39.63)	4.6	(15.98)
Mg	48.5	32			0.6	0.45	0.5	$MgSO_4$(23.62)	0.21	(15.69)
Fe	10.6	5			2.07	1.55	2.6	FeEDTA(5.78)	0.72	(3.11)
Mn	1.97	0.5			0.09	0.06	0.07	$MnSO_4$(0.77)	0.03	(0.19)
Cu	0.07	0.07			1.32	0.99	1.07	-	0.46	-
Mo	0.05	0.05			1.92	1.44	1.55	-	0.66	-
Zn	0.2	0.2			1.02	0.77	0.82	-	0.35	-
SO_4	16.2	10.0			15.3	11.48	12.3	-	5.29	-
B	0.3	0.3			0.36	0.27	0.28	H_3BO_3(0.011)	0.12	(0.10)
Na	-	-	Weight of Sodium Chloride added:		18	13.5	14.5	-	6.2	-
Notes.	Unchanged	Unchanged	3.66g	1.56g	DOPE added to water to raise soln. conductivity to 2500mmhos cm^{-2}. 19.57g K_2SO_4 added to give K:N ratio of Libsol(Starter) 14.68g K_2SO_4 (make-up).		DOPE diluted with water until K level is the same as libsol. Nutrient shortfalls are made up with salt additions so the K-Rel treatment resembles the Libsol. P is added in the form of H_3PO_4 used for pH control. $SO_4^{=}$ is supplied by anions from other salts.			
			Sufficient sodium added to match the Na levels in the K relative treat.							

All values in mg/L.

of effluent added to the solution. The DOPE with added nutrients treatment make-up comprises 10.38 litres of effluent plus the requisite additions of nutrients. The pH of the solution was maintained at 6-6.5 by the addition of phosphoric acid, and conductivity between 2 and 3 mmhos/cm.

8.2.3.Experimental.

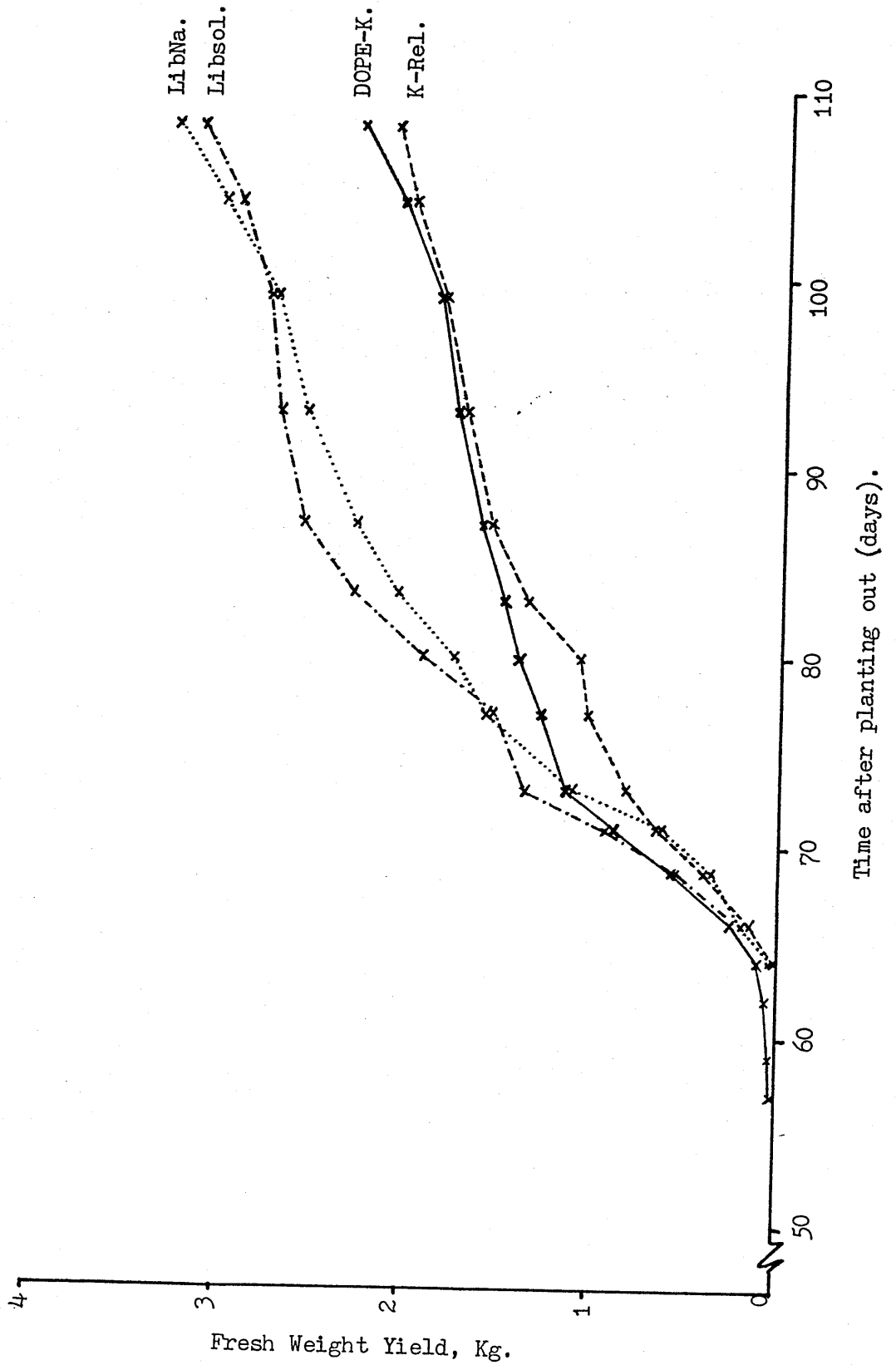
Tomato plants (Lycopersicon esculentem,cv Alicante) were obtained from a local nursery when approximately 15cm high. These were planted out into 10cm whalehide pots containing John Innes No.2 potting compost, and placed in the runs, 10 plants per run. The plants were grown to 5 trusses, and fruit picked when ripe. The fruit was weighed, examined for blossom end rot, and classified by EEC class standards. The "superior" grade was not used as fruit are allocated to that class entirely on qualitative criteria.

Fruit samples from each treatment were taken and the calcium content determined. In addition, leaf samples from the fourth leaf cluster from the top of the plant were taken and calcium, potassium, sodium and phosphorus concentrations determined. Statistical testing followed the ranked t-test method, for the yield data. For the values scored in percentages, and the leaf analyses, however, Bartlett's test for homogeneity of variance was not significant, and the non-parametric Mann-Whitney U-test was used for the non normally distributed samples.

An attack of Phytopthora parasitica was suppressed with an application of AAterra (Midox,Ltd.,Altringham), and whitefly infestation controlled with Encarsia formosa.

All solutions were discarded after 20 days and replaced with fresh, start-up compositions. Approximately 40 days after planting out, and immediately following a change of solution, samples of sump liquid were taken at daily intervals and analysed for ammonium nitrogen, using an EIL ion specific electrode.

Graph 8.3.a. Cumulative Mean Fresh Weight Yield of Tomatoes, by Treatment, against Time.



8.3. Results.

8.3.1. General.

The attack by the fungus Phytopthera parasitica was suppressed with an application of AAterra. The plants which had shown symptoms of attack-wilting, root rot and stem base lesions were not included in the analysis. 5 plants from each treatment were affected with the exception of the DOPE-K treatment, all the plants of which remained healthy. Five plants from this treatment were randomly selected, and excluded from the analysis.

8.3.2.Yields.

Graph 8.3.a. shows the mean fresh weight yield for all treatments, over time for the duration of the experimental period, demonstrating the overall yield differences between the treatments. After an initial period where all yields appear comparable, there is a relative decline in yield in the effluent based treatments. Table 8.3.b. gives a breakdown of the means of overall yield, average fruit number, and plant weight. As is the case with all tabular results presented in this experiment, the measured parameters are grouped by statistically significant subsets at the $P<0.05$, indicated by a superscript letter.

As is reflected in the graph, there is no difference in yield between the commercial treatments, or between the effluent based treatments, but both subgroups are significantly different. Similarly, there is no significant difference between the commercial solutions, or the effluent based solutions, respectively, in the average number of fruit per plant, but the potassium relative (K-Rel) treatment is significantly lower than both the commercial solutions, while the DOPE-K treatment is not. A more complex picture emerges from the average fruit weight values: the libsol plus sodium (LibNa) is significantly greater than both the effluent treatments; and while there is no difference between Libsol and LibNa, Libsol is not significantly different to K-Rel, although it is to DOPE-K.

Table 8.3.b. Total Weight of Fruit, Fruit Number, and Average Fruit Weights for all treatments. Mean of 5 plants.

	Libsol	LibNa	K Rel.	DOPE-K.
Total Weight of fruit.	3133(106)^{a}	3268(118)^{a}	2098(133)^{b}	2279(323)^{b}
Fruit Number.	46.1(2.0)^{a}	42.7(3.7)^{a}	33.2(1.8)^{b}	42.5(5.4)^{a,b}
Average Fruit Weight.	67.9(2.7)^{a,b}	76.5(5.2)^{a}	63.2(2.0)^{b,c}	53.6(4.0)^{c}

Brackets indicate standard errors of the mean.

Superscript letters within rows indicate homogenous subsets at the $P<0.05$ level.

Table 8.3.c. Percentage of Fruit in EEC Classes (by weight), Total Weight of Marketable Fruit, and incidence of fruit with Blossom End Rot and Greenback, by numbers. All Treatments. Mean of 5 plants.

	Libsol	LibNa	K Rel.	Dope-K.
% of fruit per class by weight.				
Class 1	89.2(2.2)^{a}	59.8(9.5)^{b}	82.3(13.4)^{a}	50.5(10.6)^{b}
Class 2	7.2(1.8)^{a}	24.4(5.4)^{ab}	7.2(7.2)^{a}	23.5(6.5)^{b}
Class 3	1.1(0.7)^{a}	1.5(0.9)^{a}	4.8(2.5)^{b}	7.4(2.5)^{b}
Ungraded.	2.5(1.0)^{a}	17.3(3.4)^{ab}	5.7(4.2)^{a}	18.6(4.8)^{b}
Weight of Marketable Fruit	3073(91)^{a}	2781(194)^{a}	2034(152)^{b}	1825(213)^{b}
% of fruit with BER	1.9(0.9)^{a}	11.7(4.7)^{b}	0.0(0)^{a}	36.0(10.1)^{c}
% of fruit with Greenback.	3.0(1.3)^{a}	16.1(5.6)^{b}	15(15)^{a,b}	5.3(2.3)^{a}

Numbers in Brackets are Standard Errors of the Mean.

Superscript letters within rows indicate homogenous subsets at the $P<0.05$ level.

Table 8.3.d. Selected Nutrient Concentrations in Leaves, and Calcium Concentrations in Fruits.

	Libsol	LibNa	K Rel.	Dope-K.
Foliar Concentrations.				
Calcium (%)	.378(0.16)^{a}	.441(0.09)^{b}	.326(0.08)^{c}	.229(0.13)^{d}
Phosphorus (%)	.719(.074)^{ab}	.770(.035)^{a}	.638(.072)^{ab}	.556(.093)^{b}
Potassium (%)	3.35(0.48)^{a}	3.47(0.25)^{a}	3.28(0.33)^{a}	4.61(0.47)^{b}
Sodium (%)	.553(0.12)^{a}	.340(0.03)^{ab}	.283(0.04)^{b}	.344(0.07)^{ab}
Fruit Concentrations.				
Calcium (mg. Kg^{-1})	329.7(11.5)^{a}	333.5(10.1)^{a}	307.4(23.3)^{a}	290.7(33.2)^{b}

All values on a dry weight basis.

Numbers in brackets are standard errors of the mean.

Superscript letters within rows indicate homogenous subsets at the $P<0.05$ level.

8.3.3. Marketable Yields.

Table 8.3.c. shows the percentages of fruits by class (on a weight basis), and total weight of marketable fruit. The trend that was set with overall yields is unchanged for saleable yield. The two commercial treatments give a significantly greater yield than the effluent treatments. Some differences by class are apparent, however. The LibNa and DOPE-K treatments have a significantly lower percentage of fruit of class 1 quality, compared to the K-Rel and Libsol treatments. In the case of DOPE-K this was due to the high incidence of blossom end rot (BER), and cracking, while for the LibNa treatment the low percentage was due to BER and a high incidence of of greenback. (For the LibNa experiment the high incidence of BER may be ascribed to moisture deficit:- approximately 35 days after planting out it was observed that a rapid depletion of sump liquid was occurring with this treatment. Subsequent investigation indicated that the plastic layflat had split, allowing nutrient solution to escape, causing moisture stress which in turn gave rise to blossom end rot.

8.3.4. Nutrient Concentrations.

Table 8.3.d. shows the foliar concentrations of calcium, phosphorus, potassium and sodium, together with calcium concentrations in the fruits, for all treatments. The fruit concentration of calcium for the DOPE-K treatment was significantly lower than all other treatments. In the case of the foliar nutrient concentrations, the implications are less clear: all treatments had significantly different calcium concentrations, while in the case of phosphorus the LibNa, and DOPE-K treatments were significantly higher, and lower, respectively, than the others. Potassium was significantly depressed for the DOPE-K treatment, all the others being non-significantly different. The highest level for sodium was the libsol treatment, which received sodium only through tapwater additions to the solution and routine spraying, while the lowest levels were recorded for the K-Rel treatment, which received sodium from the effluent.

8.4. Discussion.

8.4.1. General.

It is assumed that the attack by whitefly was uniform, throughout the treatments. The removal of the plants affected by Phytopothora parasitica, and the reduction in sample number from the unaffected treatment resulted in a sub-optimal number of individuals for statistical testing. The central limit theorum dictates that a sample of 10 individuals is required to give an approximation of the scatter occurring in a Normal distribution (Campbell, 1974). However, Bartlett's test for homogeneity of variance was not significant for the yield data, and the t-test was employed.

It has also been assumed, both in this experiment and in previous hydroponic work, that there is no variation in edaphic conditions within the polythene tunnel. This is an important condition, as the t-test assumes a random sample which, for agricultural research, is usually generated by a randomised block design. As the plants of a single treatment in NFT are physically close together, simply because a single gulley containing the nutrient solution is used, a randomised block design is impossible. If the polytube is assumed to be uniform in terms of light, temperature, etc., however, there is no neccessity to randomise the distribution of the plants.

8.4.2. Yields.

Both effluent treatments showed significant yield reductions, compared to Libsol. There was no significant difference in overall, or marketable yields between the two effluent treatments, although the unmodified DOPE-K treatment had significantly greater incidences of fruits in EEC class 2 and ungraded, with a concomitantly lower proportion of fruit in class 1.

The image of the closeness in yield pattern of the two effluent treatments shown in Graph 8.3.a. belies some important differences. The average fruit weight of the K-Rel treatment was not significantly different to the Libsol treatment, while the DOPE-K was. In addition, the

average fruit number of the DOPE-K treatment was not significantly different to the libsol, while the K-Rel was. The mechanisms causing the non significant differences in total yield for the two effluent treatments appear to differ.

For the DOPE-K treatment, calcium, magnesium, iron, manganese (and phosphate) were deficient. Russell (1973) among others has pointed to the cumulative effect of multiple nutrient deficiencies on growth and yields, when no indication of deficiency was apparent. The effect of the residual organic solutes must also not be discounted.

The case of the K-Rel treatment, where all plant essential nutrients are made up to match libsol, a nutritional explanation is insufficient. Furthermore, the low fruit number suggests a developmental (physiological) limitation (Milthorpe and Moorby, 1974). This may be attributable to the effluent, but it seems unlikely, in view of the higher fruit number for the other effluent based treatment. It remains to suggest a cumulative relationship between the added nutrients, and the effluent. Garraway's (unpublished) work on tomatoes grown on aerated piggery waste showed no (apparent) differences in yield, fruit number or fruit weight between effluent and inorganically grown plants. In that work, however, the inorganic solution was matched to the effluent, rather than the other way round. Finally, the possible impact of the fungal invasion in ostensibly healthy plants cannot be discounted.

8.4.3. Nutrient Uptake.

The addition of sodium appears to have an impact on the incidence of greenback, as there is a significantly greater percentage of affected fruit in the LibNa treatment. Greenback is caused by low potassium status or excessive sunlight or heat. Added sodium is possibly taken up in competition with potassium (sodium may act as a replacement for potassium). Curiously, the K-Rel treatment with identical sodium status did not display a significantly greater incidence of greenback than the Libsol treatment. The low incidence of greenback observed in the DOPE-K treatment, which had the highest sodium status, may be explained by the very high potassium level, caused by the need to balance the K:N ratios.

The sodium concentrations in the leaves do not reflect the treatments: this is perhaps due to the presence of sodium in the tap water, used for spraying the plants, and to make up the evapotranspiration losses from the nutrient solutions. The faster growing plants require more water, and hence receive more sodium than the slower growing treatments. Sodium has not had any effect on total fruit weight, fruit number, or average fruit weight, when comparing the Libsol and LibNa treatments.

The possible impact of potassium on the incidence of greenback has been mentioned. There is no significant difference in foliar concentrations of potassium for any of the three 'equal nutrient status' treatments, while the DOPE-K treatment is significantly higher. The most obvious explanation is the high level of potassium in the nutrient solution. While the K:N ratios of all treatments are the same, the absolute potassium level for this treatment remains very high. Steiner (1980) has suggested that uptake of cations by plants in recirculating solutions will occur in strict ratios. Potassium is exceptional in this respect. The mechanism for potassium uptake is almost entirely mass flow, and luxury K uptake is common in plant-nutrient systems where there is a surfeit (Scott-Russell,1976).

There is no significant difference between either of the effluent treatments, which had low levels of P (which was assumed to be made up by H_3PO_4 additions for pH control), and the Libsol treatment. In all cases phosphate levels in shoots were normal, and variations in levels may be ascribed to the volume of acid required to reduce pH, which itself was partly dependent on water requirements (the water supply was the same as that used for the earlier work).Phosphate levels in NFT are not, as has been pointed out, critical provided levels in excess of 10ppm are maintained.

8.4.4. Calcium and Blossom End Rot.

The physical blackening of the fruit end in developing fruits is caused by a lack of cell wall integrity invoked by the inability to form calcium pectate. The increased cell permeability, especially towards ions, which follows results in the disorganisation of membranes and organelles, and

finally massive oxidation (Ward, 1973). The gross causes of blossom end rot have been understood for some time. Maynard, Barham and McCombs (1957) refer to moisture stress, high osmotic pressure of nutrient solutions, the application of fertilisers high in potassium, the use of ammonium-N as a nitrogen source, and insufficient amounts of calcium in solution.

More recent work has attempted to quantify variations in nutrient, and organic acid concentrations within the fruit itself (DeKock et al, 1982), and suggests that a component of BER is a genetic propensity. Similarly, Maclon and Sim (1980) have demonstrated the need to investigate more fully the relationship between electrical potential difference in the plant (xylem) sap, and the nutrient solution itself, contending that a change from anionic to cationic nitrogen species 'radically disturbs' the favourable balance between ionic species in solution. More detailed and complex studies have started to trace back the biochemical pathways in the fruit itself from the origins of BER by enzyme activity studies (DeKock et al., 1980).

The previous work (Appendix III) emphasised, at a purly agronomic level, the role of ammonium nitrogen, and an absolute lack of calcium in causing BER, both of which causes are widely acknowledged as important (Pill,Lambeth and Hinckley,1978; DeKock et al., 1979). An obvious cause of BER under these experimental conditions is the presence of ammonium nitrogen as the predominant nitrogen species. The test for the presence of ammonium-N in the samples taken from the nutrient solution indicated that NH_4-N disappeared after 48 hours, indicating that a nitrifying microflora had built up in the NFT run itself (C.Chumley, pers. comm.). This does not preclude the possibility of ammonium nitrogen persisting in the nutrient solution earlier in the experiment before the generation of a nitrifying capability, bringing about BER. The lower incidence of BER in the K-Rel treatment may be explained by Feigin et al's (1980) observation that BER is related to the level of ammonium nitrogen in solution. Furthermore, Pierpont and Minotti (1977) indicate that tomatoes supplied with supplementary $CaCO_3$ display restricted ammonium-N uptake.

The low level of calcium found in piggery waste, exacerbated by the high pH of the oxidised effluent causing the precipitation of calcium

(Chapter 6), may also have contributed to the high levels of BER in the DOPE-K treatment. Notwithstanding the contributions of calcium made by the additions of tap water to the nutrient solutions, and routine spraying, the level of calcium in this treatment are very low (table 8.2.2.a.). Against this, however, is the evidence from the previous work which indicates that, on aeration, the incidence of BER is significantly reduced relative to nutritionally identical, unaerated treatments.

High concentrations of monovalent cations in plant nutrient solutions are also known to induce blossom end rot. Gerard and Hipp (1968) found higher incidences of BER when high potassium fertilisation was employed, while DeKock et al (1979) found significantly higher potassium to calcium ratios in BER affected fruit, a condition occurring in the DOPE-K treatment leaves analysed in this work.

One of the problems with blossom end rot, and attempts to associate the phenomenon with any given environmental, nutritional or physiological variable, is that its occurence is merely recorded, and no more detailed observation is made: Pill, Lambeth and Hinckley (op. cit.) succinctly describe the difficulties of working in this area:-

> "Apart from the difficulties in interpreting nutritional and water stress values, the fact that BER is a qualitative phenomenon, implies that quantitative plant parameters are merely associative and not causal."

It is apparent that much work remains to be done on BER before the causal mechanisms are tied down. For this experiment, conclusions have to be generalisations. Certainly high potassium status has resulted in BER, and the solution used to feed those plants has (initially) a very high ammonium nitrogen concentration, while the Ca status of the DOPE-K treatment was low. In practice, the high incidence of BER in this treatment is probably due to all these factors.

8.5. Conclusions.

The role of low calcium, and high potassium status, coupled with nutritionally based causes of yield restriction in the DOPE-K treatment would appear to indicate that the ratios between the nutrients in the effluent were too great a departure from the theoretical optima, bringing about a decline in yield quality for the DOPE-K treatment. Electrical conductivity, on its own, therefore, is an insufficient measure of nutrient status for this non-standard effluent liquid. This experiment does not answer all the questions posed on the use of effluents in recirculating hydroponic systems. It is apparent, however, that the effluent may not be used without nutritional modification in this context, and that sodium does not present a problem. Yield reductions do occur if the effluent is used, despite the matching of the plant essential nutrients (although it would be desirable to carry out more extensive experimentation into the cause(s) of this limitation.

Furthermore, these yields were obtained when using 10litres of nutrient solution per plant, approximately 5 times that conventionally employed in NFT (Cooper, Spensley and Winsor, 1978), a strategem adopted to prevent the build up of 'ghost' electrolytes in the DOPE-K treatment. This was coupled with flushing every 20 days, which in itself is wasteful.

It would appear that such an effluent would be unsuitable for general application to high value crop production by hydroponics, as a yield reduction of approximately 30% on conventional cropping could be anticipated. An effluent-based system may be economically viable, however, if an economic rent could be gained by marketing the produce as 'organic'.

9. DISCUSSION AND CONCLUSIONS.

9.1. Experimental.

The experimental work on tomatoes and fish has yielded useful information on the potential of anaerobically digested animal wastes for further food production. The analysis of digested, compared to undigested piggery wastes has revealed that digestion changes the physical and chemical characteristics of the slurry, particularly in the increase of high crude protein content solids of low particle size, ascribed as single cell protein or their derivatives. It has been suggested that these suspended solids, and the aqueous phase may potentially be economically significant components of anaerobic digestion as a farm based process.

The experiments reported here, however, only go some way into the possibility of adding value to ostensibly waste materials. The experimental programme has several limitations. The yield restrictions recorded for the tomatoes grown on the aerated slurry, when unsupplemented, and when augmented with additional nutrients, were arguably due, in part, to the presence of ammonium nitrogen in the recirculating solution. This experiment, furthermore, was carried out on effluent which had been removed from the holding lagoon at the farm, rather than from the digester itself.

Although the protein substitute performed well, compared to conventional protein sources, in the second fish nutrition trial, it would be desirable to capitalise on this work with subsequent investigations on the impact of varying conventional dietary components, and increasing substitution levels. The problem of copper accumulation in the extract, and consequently in the fish requires further attention.

The oxidation process was intended both to remove the oxygen demand from the liquid, and generate a solid, both of which are suitable for their

respective uses. It is apparent that the technique is yet to arrive at a sufficiently efficient state, partly in terms of the effectiveness of solid removal (which, although not an important consideration within this framework, ultimately needs consideration), but mainly as a means of generating 2 distinct materials which are suitable for application to an appropriate food production enterprise.

The lack of experimental depth may also be pointed to as a limitation; no single aspect of the system has received a comprehensive analysis in this work. While some information has been gained in the hydroponics, fish culture, and aeration work by successive refinements, all three areas of investigation have fallen short of an approach to a comprehensive description of the system. While it would be unlikely that any project of this scale would be likely to arrive at a 'comprehensive description,' it may be safely argued that any given component of this work has not yet been thoroughly explored.

These features of the thesis are due to the methodological approach selected. While the separation/aeration work is, in some senses, the central tenet of the thesis, it relies on the application of the products to food production, and is dependent on the speed of development of the slowest component of the whole system.

9.2. Method.

The early parts of this work have demonstrated that there are two ways of viewing the problem of the utilisation of organic wastes. One approach is to consider the entire food production enterprise (the farm) as a system, and by matching the different fractions of the waste materials to potential uses, generate a simple qualitative model of waste use for food production-'Bioplex'. At the other end of the spectrum, work has been carried out in order to establish whether the proposed applications of animal wastes to food production operate in practice. The two approaches are not incompatible.

There are two particularly good sets of work in the field of animal waste utilisation, cited in Chapter 2. The first is that of Garraway at Wye

College, who has used aerobically treated (not previously anaerobically digested) pig effluent as a medium for hydroponic plant production. The facets of this work which are interesting are the realisation that the method of preparing the material- the oxidation stage- is an important component of the generation of a hydroponic medium. The oxidation stage was conceived and experimented on with the express ambition of subsequently using the slurry in a food production context. This represents a departure for work in hydroponics based on wastewaters, which prior to that had taken the liquid resource as found and used it. The aeration work is coupled with a very thorough piece of work which attempted to quantify the potentially phytotoxic compounds present in slurry treated by different means.

In the area of using anaerobically digested slurries as animal feeds (specifically for ruminants), the best and most comprehensive experimental cycle was carried out by the team of Prior, Hashimoto and Chen at the Animal Experimention Station in Nebraska. The materials extracted from digested animal wastes have been tested by in vivo experimentation, which gave rise to ash, energy and nitrogen utilisation coefficients. Companion experiments on the chemical characteristics of the extracted material have completed the profile. This feeding work was augmented with consideration for the separation technique involved in generating the feed material, with the recognition that it is an important component of the system (i.e. that a relationship exists between the value of the feed and the means of producing it).

What both sets of work have in common is that the specific application to which the liquid (or solid) will be put has some bearing on how the material is generated. Furthermore, the experimental work must be iterative: the initial experimentation on the generation of the material is followed by the biological validation of its value. The conventional experimental framework does not offer a comprehensive method of research in this area, and the experiment on the plants or animals must be considered as part of the system.

Conversely, from the Bioplex standpoint, it could be argued that this approach is limited: the waste materials are considered in terms of their

suitability for the particular application; the fractions generated by a separation/aeration technique are seen as either useful, or not useful. The attempt to utilise the ostensibly waste animal slurry generates another waste product.

The Bioplex approach is, of itself also insufficient: recognition of the relationships between the fractions of the animal waste and possible applications does no more than pose the problem.

Both the generation of models of possible waste use, and normal experimentation on these uses are neccessary. In a problem of resource allocation, the relationships between (say) the means of generating the feed material suggested in the model, and the actual result of feeding the material must be recognised experimentally. The consequence of this statement is that the final food production application must be considered when the idea of waste utilisation is first conceived. While the converse approach of applying a resultant material from a treatment process may be successful (for example the refeeding of the coarse solids generated by the separation of untreated cattle slurry), it is desirable to make a clear statement of objectives at the outset.

This approach is a bastard version of a technique more commonly employed in operational research. Jenkins (1972) descibes this approach to problem solving as the formulation of objectives, organisation of the project, definition of the system, objectives of the system, and information and data collection. Jenkins does, however, place great emphasis on 'model building', which may be attempted on the basis of conventional reductionist experimentation on each of the components (Spedding, 1979). The experiments in this work demonstrate that this approach is limited.Systemic experimentation, such as is undertaken here, emphasises the relationships between the component areas of the system. (r)

9.3. Results.

On the basis of the second fish trial, and the hydroponics experiment employing the oxidised liquid, the protein extract is worth 105 pounds per dry tonne, based on a direct comparison with the value of soyabean meal, while the value of the liquid may be estimated at 8 pounds per M^3 of liquid, based on the value of tomatoes in 1983, and assuming no benefit from selling the produce as 'organic'. These values are made on the basis of the yields, and the upstream costs of preparation of neither the solid nor the liquid are considered. Furthermore, estimates of the amount of either the solid or the liquid for a given volume of slurry may not be estimated, due to the lack of development of any viable separation technique.

9.4. Discussion and Conclusions.

Within the framework of the approach, the work gives some notion of the value of the resultant materials. More work is required in all three areas of investigation, and a pilot scale activated sludge process with enforced aeration, but with restricted access to the open air, coupled with initial pH reduction, followed by a settling tank and an active separation process would generate both a solid and a liquid appropriate for use as a substitute feed material and hydroponics medium. It seems likely, however, that the single cell protein extract would prove more valuable than the liquid (if the liquid were used for hydroponics), given the high capital and running cost of horticultural enterprises.

The method employed in this work has both advantages and drawbacks. Because of the need to link the separation experimentation with subsequent trials, the effluent from the lagoon, rather than from the digester was used for the hydroponics work, as a homogenous liquid was required for the duration of the experiment and, at that time, the effluent taken direct from the digester had not been subjected to aeration. Similarly, the inefficiency of the second aeration rig in producing the protein extract resulted in only sufficient material for one fish trial being generated.

The difficulties experienced in this experimental work, however, give some indication of the problems which may be experienced with practical applications. If a Bioplex approach is taken to the problem of efficient utilisation of animal wastes on farms, the inter-dependence of the elements of the system may lead to inefficiencies due to the failure of a single component. The economic benefits due to diversification ascribed to this approach need to be weighed against the possible pitfalls of constructing a series of apparently autonomous food production enterprises which ultimately rely on the effective operation of the whole.

APPENDIX I. MATERIAL CHARACTERISATION.

I.1.Introduction.

A great deal of work has been done on the changes in the form of carbon present in animal wastes subjected to anaerobic digestion, simply because the fate of organic carbon determines the efficiency of the digester in terms of methane production. Another feature of anaerobic digestion, however, is that, with the exception of small losses of nitrogen as gas, all the nutrients in the slurry are conserved. This is important if the slurry is to be used in subsequent food production enterprises.

One aspect of the 'Bioplex' proposals mentioned in Chapter 1 is the use of different fractions of digested slurry in food production systems which are best suited for their use. It is therefore desirable to establish the partition of nutrients in the slurry, and the effect of digestion on partition. Nitrogen is focussed on as the most important.

I.2. The farm.

While it would be possible to carry out the anaerobic digestion of animal wastes on a laboratory scale, it is desirable, where possible, to establish the in vivo characteristics of raw and modified slurry. To this end, an operational farm digester was sought out.

Botany Bay Farm, Hitchin, Herts., is a small pig farm, producing 2000 porkers a year.The farm has 110 sows, half of which are on straw, the other half being on slats in the farrowing house. In addition 240 porkers and 140 growers (35-45 lb) are on slats at any one time. The slurry from the animals on slats is centrally collected and used in the digester. These animals produce a total of 600-700 gallons (2.72-3.18 M^3) of slurry a day.

In 1978 the increasing size of nearby Hitchin caused the farmer, Mr. J. Hart, to confront the problem of odour nuisance. After initial experiments with aeration, the decision was taken to install an anaerobic digester as a pollution control measure. A modular design, by Farm Gas, was chosen. This comprised 3 units of 2500 gallon ($11.34M^3$) capacity, giving a total size of 7500 gallons ($34.02M^3$). The slurry has a residence time of 12 to 14 days. The resultant biogas is used for heating the digester via water and heat exchange, and the surplus is used for cooking and space heating in the farmhouse. Photograph I.2.a. shows the digester, and the process of abstracting digested slurry from the third of the three modules, while photograph I.2.b. shows the lagoon behind the digester.

The pigs are on a dry feed regime, comprising 50% wheat, 25% barley, with the remainder made up of skimmed milk powder, oil, fish meal, soya bean meal, and minerals. The computed copper content of the diet is 200ppm. Not included in this computation is the copper which has been added by the EEC to the skimmed milk powder. The digested slurry is pumped into a holding lagoon and thence spread on the grassland of a neighbouring farmer.

I.3. Methods.

Undigested slurry was collected from a reception tank which receives effluent directly from the slatted floor pig houses. The slurry was removed from this tank by the pump which serves the digester, the flow being diverted to facilitate collection in 50 litre capacity plastic tubs. Digested slurry was initially collected from the outfall pipe feeding the lagoon, by bucket, eventually filling the larger tubs. Later on in the experimental period, an access port was cut into the outflow pipe, and digested slurry was removed by syphoning directly from the third (and final) module of the digester.

The 50 litre samples were thoroughly mixed, and samples taken during this agitation. Particle size distribution was established by passing a known volume of effluent through a series of sieves, of declining mesh size. The solids from each sieve were thoroughly washed with a known

Photograph 1.2.a. The anaerobic digester which was the source of the experimental material, and the process of syphoning slurry directly from the third module.

Photograph I.2.b. The holding lagoon behind the digester at Botany Bay Farm.

volume of distilled water, while the sample was still in the sieve. The washings were retained, added to the original sample, and the procedure followed with the next sieve. The smallest sieve used was 53μms. The weight of solids below this size were determined by taking subsamples of the residual liquid and washings (thoroughly agitated), and passing them, successively, through Whatman 541 hardened filter paper (25μms), Whatman GF/C glass microfibre filter paper (1.2μms), using a Hartley funnel, and a Metricel membrane filter (0.45μms). The filter papers were oven dried, and weights determined. For the membrane filter, dessication by placing over silica gel for 48 hours was employed. Replicate filter blanks were oven dried to calibrate for paper moisture content.

Ammonium nitrogen was determined by steam distilling an alkaline slurry sample into a boric acid solution, and back titrating against sulphuric acid, after the conventional Kjeldahl distillation method (ADAS, 1981). Total solids were obtained by drying 1 litre slurry samples in an oven set at 102°C for 48 hours, and ash by placing subsamples of the dried slurry, homogenised by grinding to pass a 2mm sieve, in a muffle furnace at 550°C for 18 hours. Determination of pH was by electrode. Biochemical, and chemical oxygen demands were established by the standard wastewater methods (Anon, 1971). Total nitrogen was determined on oven dried samples ground to pass a 2mm sieve, or known volumes of liquid, by a modified macro-Kjeldahl method (ADAS, op. cit.)

Sedimentation was carried out by placing slurry samples in 1 or 2 litre measuring cylinders, and allowing to stand for 24 hours. Any crust that had formed was removed, oven dried and weighed. The supernatant effluent was carefully syphoned off, and the settled solids placed in a beaker, with distilled water washings to remove residual material in the cylinder, and oven dried. The solids held by muslin cloth were those solids which retained after the muslin cloth of 0.5mm pore size, was secured over the top of a bucket, and a litre of slurry gradually introduced onto the cloth. After gravity draining for 10 minutes, the muslin cloth was untied, and hand wrung for 10 seconds. The solids retained were placed in a beaker and oven dried, together with distilled water washings containing residual particles. All determinations are the means of three replicates, except COD, BOD_5, and muslin removals which are the means of 2.

I.4. Results.

Table I.4.a. shows the changes in selected parameters of the slurry brought about by digestion. Kjeldahl nitrogen, ammonium nitrogen, ash content and pH are all higher after digestion, while the total solid content has fallen by 48%. Graph I.4.b. shows the distribution of total solids in the slurry, by size against weight, before and after digestion. Digestion has the effect of appreciably reducing the coarse solid component of the slurry. Both digested, and undigested slurry show a marked break in particle size at about the 50μm level, but the digested slurry has a higher proportion of solids below this size. Graph I.4.c. shows the crude protein content of both digested and undigested solids from various size fractions. There is little difference in crude protein content in the coarser fractions, but in the smaller particles, digested slurry is richer in nitrogen.

Table I.5.a. shows the solid removal by the two separation techniques employed. The muslin cloth removed a much higher percentage of total solids in the undigested, compared to the digested slurry. Inspection of the relationship between particle size and solids weight (Graph I.4.b.) reveals that the muslin cloth removed solids to approximately the same particle size (50μm) for both digested and undigested slurry. This suggests small solids entrainment with the larger particles, as this 50μms is a factor of 10 smaller than the measured pore size of the muslin, and explains the high crude protein content of the collected solids. For sedimentation, the gap in the percentage of total solids removed for digested and undigested solids is lower, and in both cases high percnetages of solids were removed. The crude protein contents of both slurries is concomitantly higher.

Table I.6.a. shows the reduction in chemical and biochemical oxygen demand. These determinations were made on the liquids left after sedimentation and muslin extraction, as the high pollution load of the effluents neccessitate large dilutions, and if the coarse solids were left in the sample, their presence or absence in the test aliquot would cause large variations in the results. The higher BOD and COD values obtained from the liquid produced by the muslin separation, compared to

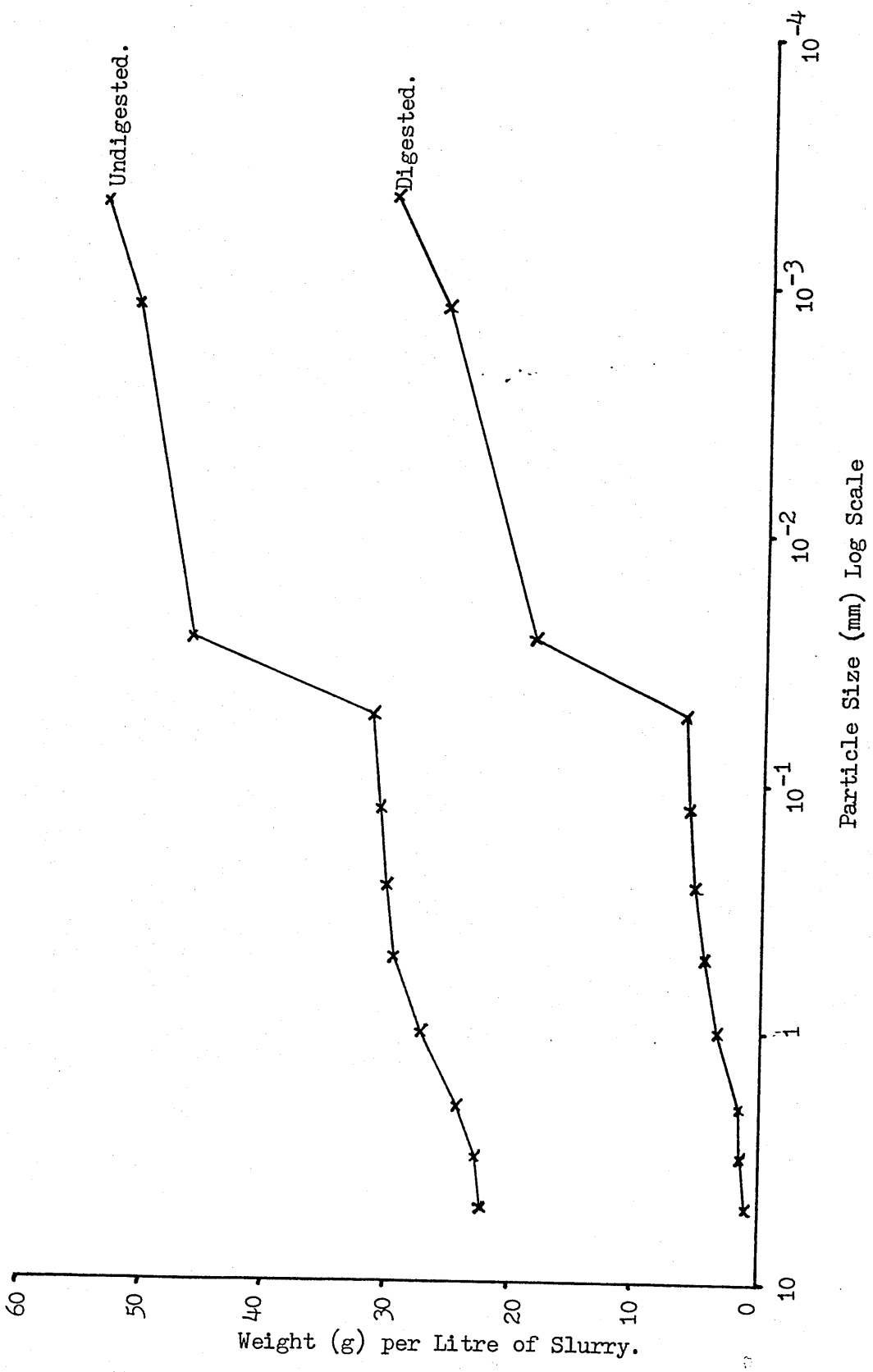
Table I.4.a. Changes in Solid Content and Nitrogen during digestion.

	Undigested	Digested
Total Solids, (%)	5.08	2.65
Ash (%)	21.03	32.46
Kjeldahl N (% dry Weight)	3.20	4.11
Ammonium N (mg. L^{-1} as N)	1051.4	1106.9
pH	7.05	8.10

Table I.5.a. Solids Removed from Digested and Undigested Slurry by Sedimentation and Filtration(Litre samples).

	Undigested	Digested
Solids Retained by:		
Muslin Cloth wt.,g.(% of Total)	32.20(63.55)	7.15(26.92)
Sedimentation; Settled (g)	36.75	18.46
Crust (g)	3.87	-
Total wt.(% Total)	40.62(79.91)	18.46(69.56)
Crude Protein Content of:		
Solids Retained by Muslin	18.62	15.80
Settled Solids	25.37	23.82

Graph I.4.b. Distribution of solids by Particle Size, Digested and Undigested Slurry (Cumulative dry weight).



Graph I.4.c. Change in Crude Protein Content of Solids by Particle Size.

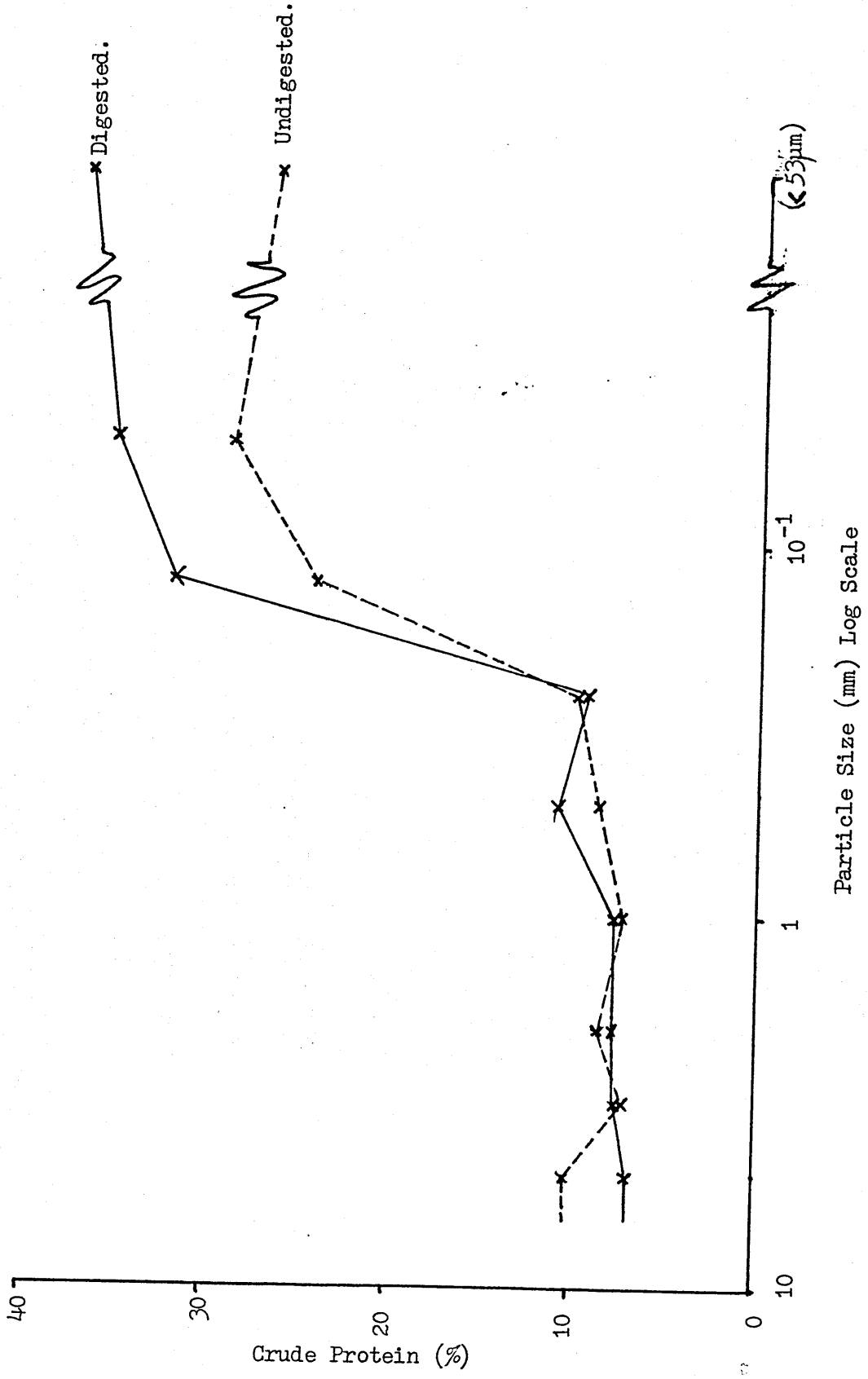


Table I.6.a. Changes in Biochemical and Chemical Oxygen Demands, by Digestion and Separation.

	Undigested	Digested
Liquid Produced by Sedimentation:		
Solution BOD_5	9500	2100
Suspended Solid BOD_5	4200	850
Total BOD_5	13700	2950
Solution COD	18800	6450
Suspended Solid COD	8600	4250
Total COD	27400	10700
Liquid Produced by Muslin Separation:		
Solution BOD_5	10600	3700
Suspended Solid BOD_5	11000	200
Total BOD_5	21600	3 900
Solution COD	19650	7650
Suspended Solid COD	32350	12750
Total COD	52000	20400

sedimentation, are consistent with the higher percentage of total solid removal achieved by the latter. The solution COD for both digested and undigested slurry is the same for both treatment. The suspended solid component of COD is greater for muslin produced liquid, due to the lower proportion of solids captured. This is reflected in the BOD values.

The ratios between the suspended solid BOD and COD values for digested, and undigested slurry change in relation to the separation. For the liquid produced by the muslin separation the ratio of undigested, to digested COD is 32350:12750 (2.54:1), while the same relationship for the liquid left after sedimentation is 8600:4250 (2.02:1). This relationship is interesting as the proportion of solids (on a weight basis) in the 'window' between the solids captured by the muslin, and those removed by sedimentation (at about the 5 x 10^{-2}mm particle range (Graph I.4.b.) is the same for both digested and undigested slurry. This higher pollution load for the undigested solids in this size range is indicative of a higher organic carbon content (and raised C:N ratio) than the equivalent from digested slurry. This observation is borne out by the higher crude protein content of digested, compared to undigested solids in this size range.

I.5. Discussion.

I.5.1. Digestion and Solids Content.

It is a truism to say that anaerobic digestion reduces the solids content of the slurry being treated. The pathways and mechanisms involved in the conversion of long chain carbon compounds to volatile fatty acids and thence to methane and carbon dioxide have been widely reported (Hobson, Bousfield and Summers, 1974; Stafford, Hawkes and Horton, 1980). Graph I.4.b. indicates that for both digested and undigested slurry a distinct break in particle size occurs at approximately 5 x 10^{-2}mm. The difference due to digestion lies in the distribution of solids on either side of this point. For undigested slurry, the percentage of total solids larger than this break point is 55%, while for digested slurry this value is about 20%. These findings are supported by work on undigested wastes

(Chang and Rible, 1975), and digested (cattle) slurry (Pfeffer, 1977).

Digestion appears to comminute and 'digest' the coarser solids, and increase the relative proportion of fine solids. The main ramification of this feature of digestion is that the proportion of solids that are amenable to removal by conventional separation techniques is reduced. Muslin cloth was employed as a separation technique for a laboratory scale simulation of farm scale separation. While the method was rather crude, the limit of particle size removal indicates that the cloth acted analogously to a viabrating screen, or filter belt press (Conway and Ross, 1981). The results from this trial indicate that most of the solids in digested pig slurry will not be captured by such techniques.

I.5.2. Digestion and Nitrogen.

The reduction in total solids by digestion leads to an apparent increase in slurry nitrogen content. In practice, nitrogen concentration on a fresh weight basis is unchanged, as there is very little difference in slurry volume (Hills and Kemmerle, 1981).

The concentration of ammonium nitrogen increases during digestion. The 5% increase in ammonium N observed in this work is relatively low: Summers and Bousfield (1980) record a 25% increase, while Shadduck and Moore (1975) in a bibliography of animal waste anaerobic digestion show ammonium nitrogen increases of up to 100% in effluent over influent values, depending on initial concentrations.

In non-ruminant animal, and plant material based effluents, plant associated organic nitrogen undergoes ammonification, and subsequent uptake and incorporation into protein by digester bacteria (Hobson, Bousfield and Summers, op. cit.; Badger, Bogue and Stewart, 1979). This ammonification is less marked in the case of ruminant animal slurries, as the proportion of organic nitrogen in the form of single cell protein in the influent slurry is higher, due to voided rumen bacteria. This change in the distribution and form of organic nitrogen is reflected in Graphs I.4.b and I.4.c. There is little difference in the nitrogen content of digested over undigested coarse solids, but a marked increase in the nitrogen content of smaller particles , especially those less than 53μms.

Undigested slurry has a greater proportion of total nitrogen associated with the coarse solid fraction, while in digested slurry most of the organic nitrogen is associated with very fine, and suspended solids.

I.6. Conclusions.

Bellamy and Hughes (1980) have suggested that approximately half the crude protein in the effluent from an anaerobic digester is in the form of microbial protein. If the nitrogen content of the smaller solids in the work is assumed to be true protein, this is a reasonable first approximation. Apart from the entrainment of fine solids in the matrix of coarse solids captured by conventional separation techniques, much of this protein remains in the resultant liquid fraction.

APPENDIX II. SOIL APPLICATION OF DIGESTED SLURRY-POT EXPERIMENT.

II.1. Introduction.

As is indicated in chapter 1, the received view on land spreading of digested slurries is that digestion enhances the plant available nutrient levels relative to undigested materials. This experiment seeks to establish the first season availability of nitrogen in digested pig slurry, compared to undigested slurry from the same source.

II.2. Materials and Methods.

A soil that receives only composted farm yard manure and basic slag (a sandy clay loam, 0.17%N, 2.1%C) was air dried and ground to pass a 2mm sieve,and samples of approximately 2kg were placed in polythene pots. Digested and undigested pig slurries, together with an inorganic control, were used in the treatments listed in Table II.2.a.

In addition, each pot received the equivalent of 40 kg ha^{-1}P (as P) in the form of calcium hydrogen orthophosphate. Each treatment had six replicates. The fertiliser application was incorporated into the top 5cm of the soil to inhibit ammonia volatilisation losses. The inorganic nitrogen treatment comprised ammonium nitrate.

The solids were obtained by filtering the slurry through a cheesecloth press, with a pore size of 0.5mm, and the liquid fraction comprised the residual filtrate. The solids were directly applied, and not aged or composted.

The pots were sown with perennial ryegrass (Lolium perenne, S23), placed in an unheated greenhouse and watered to capacity daily. The grass was sown on June 4, and cuts were taken on July 5, August 5, and September

Table II .2.a. Treatments employed in the Pot Experiment.

Complete Slurry, Undigested *
Complete Slurry, Digested
Solid Fraction, Digested
Solid Fraction, Undigested
Liquid Fraction, Digested
Liquid Fraction, Undigested
Inorganic
Control (no additions)

* All treatments received 150kg ha^{-1} equivalent Nitrogen.

13, 1981. The grass samples were oven dried, and the dry weights and nitrogen contents established. Nitrogen determinations were carried out using a modified macro Kjeldahl method (ADAS, 1981). Statistical testing followed the ranked t-test method.

II.3. Results.

Tables II.3.a. and II.3.b. show the dry weight yields, and nitrogen uptake into shoots, respectively, for each cut, together with a cumulative total for each treatment. Table II.3.c. indicates the percentage nitrogen in the shoots, by cut, for the same treatments.

II.3.1. Dry Weight Yields.

There is a significantly greater dry weight yield for the inorganic treatment for all cuts and the cumulative yield, compared to all other treatments. With the exception of undigested solid first cut, and undigested all second cut, which are both lower than their partners, there is no significant difference between digested and undigested treatments.

II.3.2. Nitrogen Uptake.

Table II.3.b. demonstrates that the largest differences in nitrogen uptake occur early in the growth period, with the inorganic treatment showing significantly greater N removal than any other treatment. Predictably, the control (no application) has a lower level of removal than any other treatment. There are significant differences between the complete slurry , and the slurry solid pairs. These uptake values set the trend for the entire experiment. In the second cut only the inorganic treatment shows a significantly greater value than all other treatments, and in the third cut no differences between any treatments emerge.

A similar pattern is seen in the values for the percentage nitrogen in the tops. There are significant differences between the 'complete' and solid treatment pairs, and the inorganic value is significantly higher than any other treatment in the first cut. For the second and third cuts no differences emerge, except that, interestingly, the %N in the inorganic

Table II .3.c. Percentage Nitrogen in Shoots, by Cut.

Treatment	Cut 1	Cut 2	Cut 3
Undigested Complete	2.47(.19)[a]	1.27(.08)[a]	0.89(.05)[a]
Digested Comlete	2.89(.13)[b]	1.23(.07)[a]	0.97(.05)[a]
Undigested Liquid	2.84(.14)[b]	1.20(.09)[a]	1.00(.05)[a]
Digested Liquid	2.70(.20)[b]	1.25(.10)[a]	0.92(.05)[a]
Undigested Solid	2.35(.35)[a]	1.22(.17)[a]	0.98(.08)[a]
Digested solid	1.83(.52)[c]	1.41(.09)[a]	0.97(.03)[a]
Inorganic	3.50(.52)[d]	1.33(.12)[a]	0.75(.06)[a]

Brackets indicate standard errors of the mean.

Letters indicate homogenous subsets at the $P<0.05$ level within columns.

Table II..3.b. Nitrogen Uptake into Shoots, by Cut and Cumulative, for all Treatments (g).

Treatment	Cut 1	Cut 2	Cut 3	Cumulative
Undigested Complete	0.109(.004)^{b}	0.040(.003)^{a}	0.063(.005)^{a}	0.212(.006)^{d}
Digested Complete	0.130(.011)^{c}	0.043(.003)^{a}	0.065(.004)^{a}	0.238(.016)^{d}
Undigested Liquor	0.144(.010)^{c}	0.041(.003)^{a}	0.072(.003)^{a}	0.257(.011)^{d}
Digested Liquor	0.132(.006)^{c}	0.045(.003)^{a}	0.067(.005)^{a}	0.244(.006)^{d}
Undigested Solid	0.094(.024)^{b}	0.033(.005)^{a}	0.058(.004)^{a}	0.185(.028)^{b}
Digested Solid	0.072(.011)^{a}	0.032(.003)^{a}	0.066(.005)^{a}	0.170(.012)^{b}
Inorganic	0.219(.012)^{d}	0.066(.009)^{b}	0.059(.009)^{a}	0.336(.016)^{e}
Control	0.074(.006)^{a}	0.036(.004)^{a}	0.057(.005)^{a}	0.166(.007)^{a}

Brackets indicate standard errors of the mean.

Letters indicate homogenous subsets at the $P<0.05$ Level within columns.

Table II .3.a. Dry Weight Yields, By Cut and Cumulative, For All Treatments(g).

Treatment	Cut 1	Cut 2	Cut 3	Cumulative
Undigested Complete	4.55(0.38)^{b}	3.19(0.21)^{a}	7.10(0.38)^{a}	14.84(0.63)^{b}
Digested Complete	4.54(0.37)^{b}	3.61(0.31)^{b}	6.69(0.26)^{a}	14.84(0.53)^{b}
Undigested Liquor	5.15(0.48)^{b}	3.52(0.27)^{b}	7.30(0.37)^{a}	15.97(0.87)^{c}
Digested Liquor	4.98(0.38)^{b}	3.71(0.35)^{b}	7.35(0.42)^{a}	15.88(0.62)^{c}
Undigested Solid	3.90(0.51)^{a}	2.73(0.27)^{c}	5.92(0.20)^{a}	12.56(0.79)^{a}
Digested Solid	4.08(0.41)^{b}	2.27(0.09)^{c}	6.79(0.39)^{a}	13.13(0.64)^{a}
Inorganic	5.56(0.38)^{c}	5.72(0.48)^{d}	8.29(0.39)^{b}	19.24(0.70)^{d}
Control	3.28(0.29)^{a}	2.73(0.22)^{c}	6.25(0.37)^{a}	12.26(0.39)^{a}

Brackets indicate standard errors of the mean.

Letters indicate homogenous subsets at the $P<0.05$ Level within columns.

treatment in the third cut is significantly lower than any other treatment.

II.4. Discussion.

II.4.1. General.

The percentage nitrogen values in the first two cuts for all treatments are in the range expected for perennial grasses (Russell,1973). The final cut shows a relatively low figure because the coarse stems close to the soil surface were included, and because lowered N content is expected in a physiologically older stand. Differences in N removal and dry weights between solid, liquid, and complete slurry treatment types is expected; the low values associated with the solid fraction of both slurries is a function of the added nitrogen being chemically associated with plant derived materials. Since these materials have passed through the anaerobic digester relatively unaltered, they are assumed to have a high lignocellulose content, reducing their susceptibility to microbial attack (Alexander, 1972). The presence of organic carbon raises the C:N ratio, indicating reduced N availability (Power and Legg, 1978). This suggests that it would be beneficial to compost the solids if a separation procedure were to be followed in field scale applications.

The liquid fraction of digested slurry is relatively high in N, having 73% of total N, compared to 58% of total N for undigested material, whilst having less carbon. This is in line with other observations on the differences between digested and undigested animal wastes (Bartlett et al.,1978, Fulhage, Porter and Fischer, 1978). Much of this nitrogen is in the form of readily available NH_4-N, the fraction of which increases with digestion (Summers and Bousfield, 1980), and approximately half is in the form of microbial protein (Bellamy and Hughes, 1980), most of which is in suspension. This is relatively accessible to plants over a growing season. The increases in these forms of N are at the epense of plant associated nitrogen (Badger, Bogue, and Stewart, 1979), which is less readily transformed to forms which are available for plant uptake (Power and

Table II .4.2.a. Percentage Nitrogen Utilisation (N removed in tops).

Treatment	% N use.
Undigested All	45.1(1.37)[a]
Digested All	50.6(3.34)[a]
Undigested Liquid	54.6(2.42)[a]
Digested Liquid	51.8(1.34)[a]
Undigested Solid	39.8(5.93)[b]
Digested Solid	36.1(2.52)[b]
Inorganic	77.8(6.68)[c]

Brackets indicate standard errors of the mean.

Letters indicate homogenous subsets at the $P<0.05$ level.

Legg,op.cit.). These factors, coupled with the reduced C:N ratio which Tietjen (1966) comments on, indicates increased N availability.

However, the lack of difference in yield and N uptake may in part be attributed to increased losses due to ammonia volatilisation, which occurs even when the slurry is incorporated into the soil (Terman, 1979). The high pH of the slurry (in this case 8.1), and the naturally calcareous soil type employed in this experiment would increase this trend. Cooke (1975) indicates that responses to N are greater with nitrate rather than with ammonium salts on calcareous soils due to this factor.

II.4.2. Nitrogen Utilisation.

Table II.4.2.a. shows the percentage nitrogen utilisation (shoots only) for all treatments. This value is uncorrected for nitrogen removed by the control (no application) treatment, and is a simple index of nitrogen applied/ nitrogen removed. The low nitrogen removal values for the solids are expected: the higher C:N ratio and greater percentage of nitrogen in organic forms associated with the plant derived material indicates reduced N availability and susceptability to microbial transformations in the soil. The remaining four slurry treatments are within the range of N utilisation expected for animal waste applications to soils (Spedding, Walsingham and Hoxey, 1981).

II.5. Conclusions.

Although the trial is pot based, and subject to the limitations of this experimental format, the results show no evidence of differences in first season nitrogen utilisation between digested and undigested piggery waste. The availability of nitrogen may be greater for digested slurry but, as is discussed above, the propensity for nitrogen losses from the system by leaching and volatilisation is concomitantly increased.

APPENDIX III. DIGESTED PIG EFFLUENT AS A HYDROPONIC MEDIUM.

III.1. Introduction.

Effluent wastewaters have been used as a medium for hydroponic plant growth, as is discussed in Chapter 2, but only in open flow systems due to the low concentrations of plant nutrients. Animal effluents, even after pollution control treatment, are too strong to be used directly for hydroponics without dilution. If an open flow system were to be used, continuous additions of large volumes of water would be required, and a more appropriate technique is closed system (recirculating solution) hydroponics. While some dilution is needed to reduce the osmotic potential of the effluent, the levels of water addition are greatly reduced.

Garraway (unpublished data) has successfully used aerobically treated pig slurry as a medium for the growth of tomatoes in a recirculating system, and found no difference in yield between the effluent medium, and an inorganic nutrient control, with similar nutrient concentrations to the effluent. No work of the same type has been carried out using digested animal wastes, however (A.Cooper,pers. comm.). This chapter deals with the initial (feasibility) experimentation on anaerobically digested pig effluent for the hydroponic growth of tomatoes.

III.2. Materials and Methods.

III.2.1. The Recirculating System.

All the work reported in this appendix was carried out on the same system. This comprised 4 similar units, each composed of two 15 metre lengths of 'Layflat' plastic channelling placed in aluminium support trays approximately 25cm. wide, with sides 5cm. high. Both the Layflat and the

aluminium trays were purchased from Soilless Cultivations Ltd., of Aldershot. The trays were housed in an unheated polythene greenhouse (polytube) with a gravel floor. From the top to the bottom end of each tray a gradient of 1 in 50 was maintained. A gap of 0.6M was kept between each tray, and the sides of the polytube. Each pair of adjacent trays drained into a plastic tank sunk into the ground so that the top of the tank was flush with the gravel floor. Each tank held 160 litres of nutrient solution. 160 litres of nutrient solution can theoretically accomodate 80 tomato plants (Cooper,1979), and at no time in the experiments did the plant number exceed 50 per tank.

In each case liquid was recycled using a Beresford PV42 190Watt pump secured above the tank. The recirculating solution was drawn up from the sump tank passing through a filter of 1mm. gauze to prevent clogging with larger particles. This filter contained a non-return valve, neccessary as the pumps needed to be manually primed. The solution was pumped to the top end of the run and introduced into the plastic channel at the rate of 600-800cm^3 per minute. Each pump served two channels, an arrangement achieved by splicing two 6mm. internal diameter feeder tubes into the main pipe. Flow regulation was by means of a two-way tap plumbed into the outlet port of the pump, allowing solution flow to the feeder hose, or returning direct to the sump.

III.2.2. The Nutrient Solutions.

In these experiments the inorganic control solution was a commercially produced Nutrient Film Technique (N.F.T.) composition, 'Libsol' supplied by Interlates Ltd., Skelmersdale. The pig effluent was the supernatant of anaerobically digested piggery waste from Botany Bay Farm, Hitchin, which had been allowed to settle for 7 days before use, facilitating the removal of suspended solids. The plant nutrient content of both the effluent and the Libsol are given in Table III.2.2.a., and more detail on the effluent is given in Chapter II-1. The effluent was diluted with tap water so that the electrical conductivity was reduced from 8.2mmhos, to 3mmhos, a dilution of one part effluent to three parts of water.Daily measurements of pH and electrical conductivity were taken for both the inorganic and effluent treatments, and nutrients were added when the conductivity fell below

TableIII.2.2.a. Compostions of Nutrient Solutions used for Hydroponics,Commercial and Experimental.

Nutrient	Long Ashton	Cooper	Libsol Starter	Libsol Replenisher	Garroway	This Work
Total N	174	200	104	49.3	69	279
NH_3-N	-	-	-	-	-	179
P (asP)	34	60	62	Nil	17.5	16
K (asK)	150	300	333	142.6	70	316
Ca	150	170	167.7	67.9	-	43
Mg	36	50	48.5	32.1	11	-
Fe	18	12	10.6	5	<.1	-
Mn	.55	2	1.97	.5	<.1	-
Cu	.064	.1	.07	.07	<.1	-
Mo	.053	.2	.05	.048	<.1	-
Zn	.066	.1	.2	.2	<.1	-
Cl	3.6	-	-	-	-	-
S (asSO_4)	11	-	16.2	10	-	-
Na	27	-	Nil	Nil	-	29
B	.56	.3	.3	.3	<.1	-

All values as mg/L.

2mmhos. Inorganic nutrients were added in the form of a Libsol 'replenisher' pack. The pH was maintained at 6-6.5, in both cases by the addition of H_3PO_4.

III.2.3. Experimental-1

On 20 April, 1982, seeds of a dwarf tomato (Lycopersicon esculentem, cv. Pixie) were placed in seed trays containing John Innes No.1 potting compost, and allowed to germinate. The resulting seedlings were planted out into 100mm. whalehide pots containing John Innes No.2 compost on 10 May. The pots were placed in the layflat runs on capillary matting at 60cm. intervals. A 30cm. stagger in plant placement in adjacent runs was maintained. 4 runs (2 treatments) were employed. As the experiment progressed, it became apparent that the effluent was supplying insufficient potassium. This was corrected by the addition of 15g. KNO_3 weekly to that treatment.

Three weeks after planting out, 3 plants from each treatment at identical positions in the runs were removed, and leaf areas, dry weights of roots and shoots, and root and shoot lengths were determined. In addition, qualitative observations of general plant condition, and leaf colour, were made throughout the growing period.

The experiment was stopped on 29 June due to power failure to the recirculating pumps, causing wilting, on the 27th. The experiment was continued at that time as it was uncertain whether damage to the plants was irreversible. After two days sufficient irreversible damage was apparent to skew the results if the experiment continued. The inorganic treatment plants were appreciably larger than those of the pig effluent treatment, and damage to the control was consequently greater due to the difference in evapotranspiration requirement. The fruit that had formed by this time were picked, and the number of trusses per plant, average weight of the individual fruit, total weight of fruit per plant, and the percentage of fruit with blossom end rot (BER) were recorded for each treatment. In addition, a mixed sample of leaves were taken from the fifth leaf cluster of plants from the effluent treatment and analysed for major nutrient content.

III.2.4. Experimental-2

The experimental rig described in section III.2.1. was used unchanged for this work. Tomato seedlings (cv.Pixie) were placed in 100mm. whalehide pots filled with John Innes No 2 compost. On 7 July 1982 the plants were placed at 90cm. intervals in six hydroponic runs, 2 runs per treatment, with a stagger of approximately 45cm. between plants in adjacent runs. The plants were placed on capillary matting. The treatments consisted of an inorganic control, and two of pig effluent. All treatments were the same as those described in section III.2.2., except that one of the effluent treatments had enforced aeration by securing the bi-pass flow rate control pipe above the surface of the liquid in the sump, rather than allowing the flow to return to the sump below the surface.

One run of each effluent treatment had no plants in the first 3 metres of the run, allowing the effluent to trickle down the layflat without competition for oxygen from plant roots, to establish whether any local aeration benefit would accrue. Additions of the appropriate nutrient source, and phosphoric acid were made to maintain the EC between 2 and 3mmhos, and the pH between 6 and 6.5, in response to daily pH and EC measurements. Every 10 days the solution in the sump was completely replaced with the appropriate fresh material. 15g. of KNO_3 was added to both effluent treatments after tank flushing, and then on the sixth day following.

Tomatoes were harvested when ripe up to 30 September. The tomatoes were weighed, examined for greenback and BER. At the end of the experiment plants grown in all treatments were measured for internode length, and leaf length and width. The results were subjected to Duncan's Multiple Range test.

III.3.1. Results, Experiment 1.

III.3.1.1. Introduction.

The results from this experiment are of three kinds: quantitative, with statistical testing, semi quantitative, with no testing, and qualitative observation.

III.3.1.2. Qualitative.

During the course of the experiment, the effluent fed plants showed clear signs of stress. This took the form of lack of full leaf development, short internode lengths, turned down leaves and leaf clusters, and short, highly branched root systems (photograph III.3.1.). These features are classical symptoms of epinasty. Early in the experiment manganese and iron deficiency symptoms developed in the same treatment (indicated by interveinal mottling of developed leaves (photograph III.3.2.), and chlorosis of the growing point, respectively (Wallace,1943)). These conditions were rectified by tight control over solution pH.

Approximately half way through the experiment mature leaves in the effluent treatment developed chlorosis at the leaf margins, followed by necrotic spotting (photograph III.3.3.). This condition persisted until the end of the experiment. Again in the same treatment potassium deficiency symptoms appeared. This problem was remedied by the addition of 15g. of KNO_3 weekly. The symptoms are shown in photograph III.3.4.

III.3.1.3.Semi quantitative.

Table III.3.1.3.a. shows the mean values of the growth parameters measured on the plants removed on 29 May. In all respects growth appears more restricted in the effluent treatment, most notably manifested in the root:shoot ratios, and leaf areas. These data must be treated with some caution, however, as no testing has been carried out, and no standard

TableIII.3.1.3.a. Mean Values (3 plants) of Growth Parameters Measured on Plants Removed on 29/6/82. Effluent and Inorganic Treatments.

Parameter	Inorganic	Effluent
Plant Height (cm.)	19.67	16.67
Plant Fresh Weight (g.)	87.38	37.80
Plant Dry Weight (g.)	9.06	4.53
Shoot Dry Weight (g.)	4.71	3.23
Root Dry Weight (g.)	4.35	1.30
Apparent Mean Root Length (cm.)	9.50	5.67
Maximum Root Length (cm.)	35.67	14.83
Leaf Area (cm^2)	756.12	462.90
Root:Shoot Ratio	1.08	2.47
Number of Flower Clusters.	7.3	4.6
Internode Length (cm.)	2.01	1.78

Table III.4.1.3.b. Nutrient Content of leaf samples from tomatoes grown on effluent, first experiment.

Element	Content (dry weight basis.)
Nitrogen (%)	6.05
Phosphorus (%)	1.34
Potassium (%)	3.42
Magnesium (%)	0.33
Calcium (%)	1.29
Manganese ($mg.kg^{-1}$)	118
Boron ($mg.kg^{-1}$)	65

TableIII.3.1.4.a. Mean Values of Yield Parameters Measured on Plants, 29th June. Effluent and Inorganic Treatments.

Parameter	Inorganic	Effluent
Total Number of Fruit per Plant.	39.6(2.33)	32.3(1.74)**
Average Weight of Fruit (g.).	14.33(0.68)	14.15(0.47)
Total Weight of Fruit (g.).	560.0(35.12)	449.7(18.73)***
% Fruit with BER.	12.1(2.0)	7.4(1.77)*

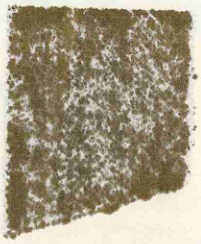
Figures in brackets are standard errors of the mean values.

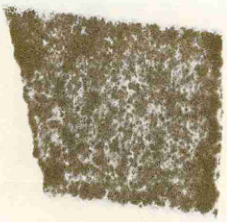
*Significantly Different at the $P<0.05$ Level.

**Significantly Different at the $P<0.02$ Level.

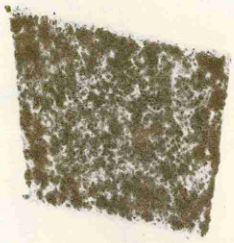
***Significantly Different at the $P<0.01$ Level.

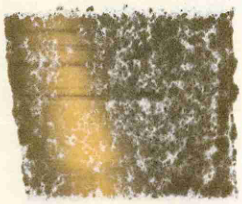
Photograph III.3.1. Young Effluent-Grown Plant showing symptoms of epinasty, attributed to phenoxy compounds present in the digested slurry. Premature abscission may be due to ethylene production.

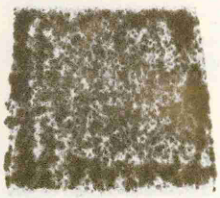


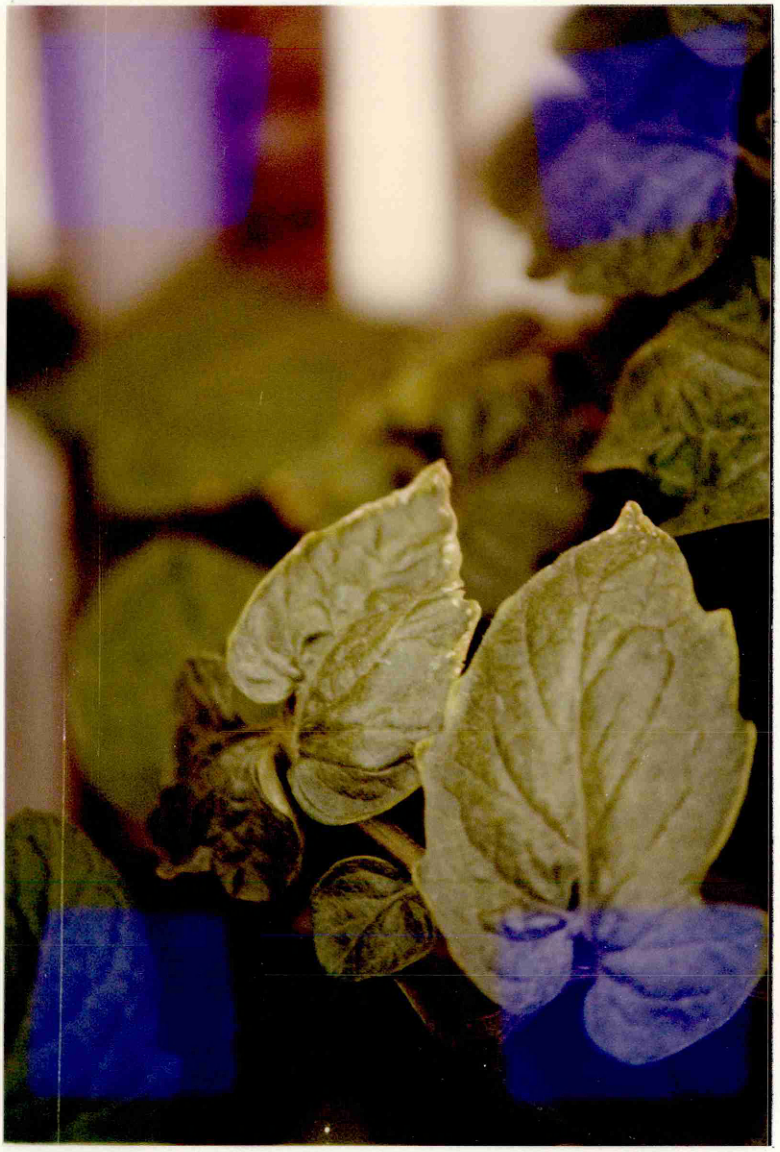


Photograph III.3.2. Manganese defiency recorded in effluent grown plants. Interveinal chlorotic spotting, giving a mottled appearance. A pH dependent disorder rectified by the maintenance of pH between 6 and 7.



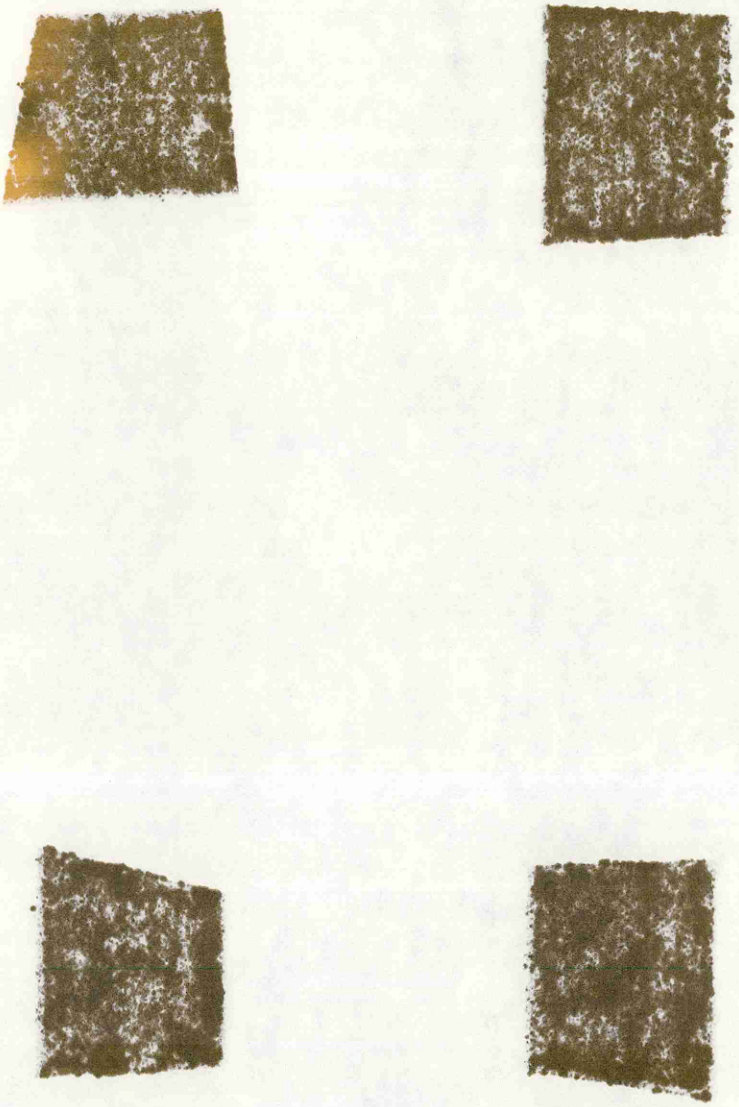






Photograph III.3.3. A nutritional disorder associated with the effluent treatment, comprising chlorosis at the leaf margins, followed by necrosis. The most probable cause is calcium deficiency, induced by high ammonium levels in solution.

Photograph III.3.4. Classical potassium deficiency symptoms: interveinal chlorosis followed by necrotic spots at leaf margins. Occurred in the effluent treatment.



errors of the mean values are offered, due to the low sample number. Nonetheless as an indication of differences in development between the treatments the results are of interest.

Table III.3.1.3.b. shows the concentrations of major nutrients in the leaf samples taken from the effluent treatment plants. The only element which is spectacularly low is potassium, while nitrogen is high.

III.3.1.4.Quantitative.

Table III.3.1.4.a. shows the results of paired t-testing between the two treatments, comparing the total number of fruits per plant, the average individual fruit weight, total weight of fruit per plant, and the percentage of fruit with BER, as measured on June 29. At this time no fruits were ripe, and all, irrespective of size, were included in the analysis. Apart from the incidence of BER, which is significantly greater in the control, and the non-significant difference in the average fruit weight, the effluent treatment gave significantly lower values than the control. This is clearly due to the low number of fruit per plant (the total weight being the quotient of this, and the almost identical average fruit weight.

III.3.2.Results, Experiment 2..

The average fruit weight, number of fruits per plant, total fruit weight, incidence of BER and greenback, by treatment, are given in table III.3.2.a. Neither of the effluent treatments show any significant differences between the runs where plants are placed at the top of the run compared to the three metre lag. Aeration increases the number of fruits per plant to the level of the inorganic control, but the low average fruit weight which is encountered in all the effluent treatments makes the total weight of fruit significantly lower than the control. As a result of the higher number of fruits, aeration increases fruit yield per plant in the effluent treatment. Aeration also reduces the incidence of BER to the level of the control, significantly lower than the unaerated effluent tretament. The incidence of greenback is significantly lower for the inorganic, compared to the effluent treatments.

TableIII.3.2.a. Yield parameters, and incidence of fruit defects, for experiment 2, tested by Duncan's Multiple Range Procedure.

Parameter	Effluent 1	Effluent 2	Effluent 3	Effluent 4	Inorganic
Number of Fruits per Plant.	9.22(1.51)^{a}	11.56(1.02)^{a}	28.00(2.80)^{b}	23.3(3.67)^{b}	24.33(2.36)^{b}
Average Weight of Fruit (g.)	33.22(3.05)^{a}	36.41(2.29)^{a}	31.86(2.13)^{a}	31.65(2.29)^{a}	55.68(3.37)^{b}
Total Fruit Weight (g.)	279.9(43.13)^{a}	429.57(56.93)^{a}	893.88(82.76)^{b}	705.13(109.23)^{b}	1034.93(88.62)^{c}
% of Fruit with Greenback.	33.36(1.97)^{a}	30.30(5.53)^{a,b}	20.48(5.98)^{b}	24.44(4.68)^{b}	8.17(1.24)^{c}
% of Fruit with BER.	15.15(5.52)^{a}	20.90(4.52)^{a}	0.78(0.78)^{b}	2.22(1.48)^{b}	5.08(2.23)^{b}

Brackets indicate Standard Errors of the Mean.

Letters indicate homogenous subsets significantly different at the $P<0.05$ level within rows.

Effluent Treatments: 1=Unaerated,No Lag,2=Unaerated,3M.Lag,3=Aerated,No Lag,4= Aerated,3M.Lag.

TableIII.3.2.b. Means of Leaf Lengths,Widths, and Internode Lengths from Randomly Selected Plants for All Treatments,Second Experiment. (Five Plants per Treatment,10 subsamples).

Parameter	Unaerated	Aerated	Control
Internode Length (cm.)	5.87(.33)^{b}	6.24(.21)^{b}	11.55(.42)^{a}
Leaf Width(cm.)	4.42(.28)^{b}	4.00(.18)^{b}	7.14(.16)^{a}
Leaf Length(cm.)	6.53(.24)^{b}	6.69(.29)^{b}	11.85(.32)^{a}

Brackets Indicate the Standard Errors of the Means.

Letters indicate homogenous subsets at the $P<0.001$ level (within rows.)

Table III.3.2.b. shows the difference in leaf size, and internode length, for the three treatments. The inorganically fertilised plants are significantly larger than both the organic treatments.

III.4.Discussion

III.4.1.General.

The low yield values, compared to normal commercial yields, and low average fruit weights from the first experiment may be explained by the enforced early completion of the experiment. The low values obtained in experiment 2 are due to the late start. The cultivar used also contributed: Pixie is a non commercial variety with an average yield of 1.5kg. per plant.

III.4.2. Non Nutritional Limitations of the Effluent.

There are several non nutritional factors which may be responsible for the yield and growth restrictions shown by the effluent grown plants.The first, and obvious, is the polluting potential of the liquid. Even after dilution with tap water, the BOD_5 of the recirculating solution is approximately 500mg. L^{-1}which exerts considerable oxygen demand in the runs, in competition with the roots. The high shoot:root ratio observed in the first experiment, together with indications of a short, well branched root system (a stress response) indicate the presence of an inhibitory factor.

In addition to oxygen depletion at the roots, certain phytotoxic compounds may be present. Garraway and Ramirez (1982) have demonstrated that anaerobic digestion maintains the concentrations of phenolic compounds present in raw animal slurry, and in addition causes the generation of other generically similar compounds. Some of these phenolic groups are known to have inhibitory effects on plant growth. An explanation of this is their similarity to auxin analogues.

Auxin or auxin analogues may be responsible for growth aberrations in plants. High concentrations of this plant hormone induce epinasty (leaf

curl, adventitous rooting), and restricted growth (Thimann,1969), and inhibition of root elongation can be the result of auxin induced ethylene production. In addition, auxin analogues may stunt or completely stop growth, halt root elongation and the expansion of young leaves, and bring about reduced internode length and lateral root initiation (Ashton and Crofts, 1973). These features have been noted in effluent grown plants, qualitatively in experiment 1, and quantitatively in experiment 2.

It is unlikely that commercial auxin analogues such as 2,4-D, or MCPA could be present in the digested slurry, but phenoxy compounds present in the effluent will act in an analagous way.

Garraway and Ramirez (op.cit.) also note that aeration of pig effluent removes phenolics. The enforced aeration in experiment 2 has improved yields compared to the unaerated treatment, but no significant differences between leaf length and width, and internode length, for the two treatments were noted, and both were significantly lower than the control in all respects. While the number of plants served by each tank is not large by commercial NFT standards (Spensley, Winsor, and Cooper,1978), that plant density requires daily nutrient additions in order to maintain the EC above 2mmhos. The somewhat ad-hoc arrangement for aeration may have been insufficient to oxidise inhibitory components in the daily additions of effluent.

Notwithstanding the lack of data, the correlation between the morphological aberrations in the effluent treatments, and the classical symptoms of auxin or herbicide induced plant damage, suggests that some of the phenolic compounds (specifically phenoxy groups) are responsible. Phenoxy compounds need not, however, be the exclusive reason for growth and yield restrictions in effluent grown tomatoes.

III.4.3. Nutritional Limitations of the Effluent.

III.4.3.1. Nitrogen.

The nitrogen in the effluent is in the form of organic N (solid and solution phase), and ammonium N in solution. The reduced conditions preclude the presence of nitrate N. Total N is 279ppm with ammonium N making up 179ppm (as N). Whilst some short chain soluble organic nitrogen is plant available (Russell,1973), most N taken up will be inorganic, ammonium N. Pill and Lambeth (1977) have demonstrated that tomatoes in sand culture fertilised with ammonium nitrogen suffer reduced shoot and root concentrations of Ca, Mg, K, and P, together with decreased water use efficiency, and increased root death compared to nitrate nitrogen fertilised plants. Feigin et al. (1980) have also shown ammonium N induced effects on tomatoes-reductions in leaf length and width, average weight of fruit, and number of fruits in hydroponically grown plants with 100% N as ammonium N. The qualitative observations are in accord with these suggestions, and average fruit weight is uniformly low for the aerated and unaerated treatments in the second experiment. The number of fruits, however, is higher for the aerated treatment.

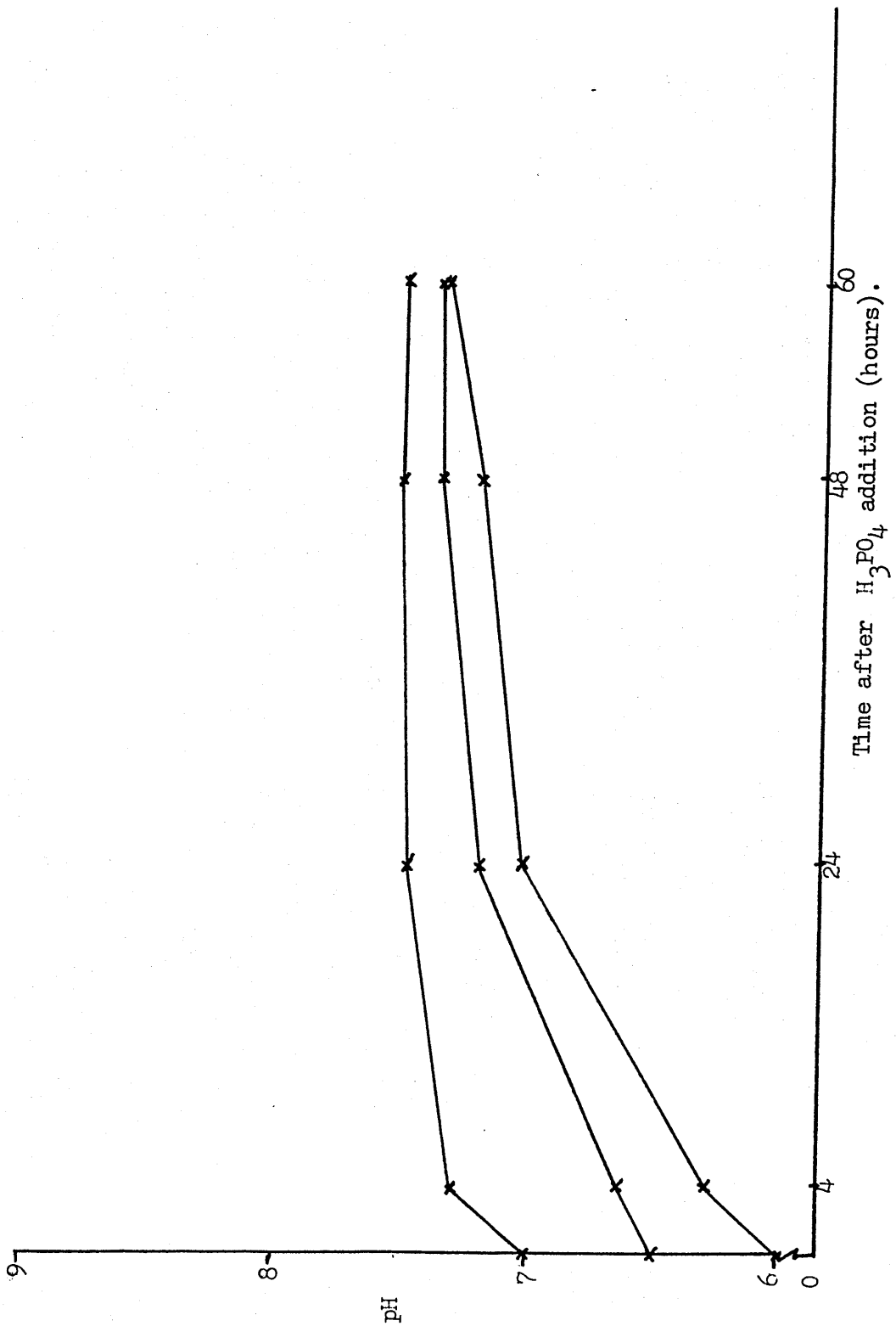
Furthermore Cooper (op.cit.) suggests that root death will occur if ammonium N exceeds 25% of total nitrogen in the recirculating solution. There is some suggestion, however, that ammonium nitrogen is readily oxidised in hydroponic systems. Cooper (op.cit.) anecdotally reports that ammonium nitrogen disappears within 24 hours of introduction, an observation supported by C.Chumley (pers.comm.). There is, however, no published evidence to support this.

It is also the case that intrinsically high levels of nitrogen, irrespective of the chemical species, can give rise to stunting, leaf curl, and death of the growing point (Massey and Winsor,1980).

III.4.3.2. Phosphorus.

Due to the high pH of the effluent, most phosphate will have precipitated. The low level of phosphorus is made up by additions of acid for pH control. P deficiency would be unlikely to occur even without H_3PO_4 additions, as tomatoes grown hydroponically are relatively insensitive to P levels above 10ppm.

Graph III.4.3.3.a. Change in pH over time of unaerated, undiluted effluent, following initial pH reduction with phosphoric acid.



III.4.3.3.Micronutrients.

The only micronutrients which expressed deficiency symptoms in the effluent grown plants from both experiments were iron and manganese. Both of these deficiencies are pH related, and were not expressed when tight control over pH was maintained. However, the nature of the slurry rendered this difficult. Graph III.4.3.3.a. shows the titration of phosphoric acid against diluted effluent, with an indication of pH changes of the effluent over time, after the addition of acid. The apparent reduction of the pH to a level acceptable for plant growth is followed by a period of buffering back to levels where micronutrients are not plant available. This buffering capacity can be attributed to the surface activity of the suspended solids.

III.4.3.4. Sodium.

The presence of sodium is likely to be a further factor in restricting growth in the effluent treatments. Sodium is present in both the effluent (Table III.2.2.a.) and the local tap water (Table III.4.3.4.a.) in relatively high concentrations. While sodium may act as a partial replacement for potassium in many plants, tomatoes included (Russell,1973), high levels are phytotoxic. Current agronomic advice (ADAS advice to growers) is to allow sodium to accumulate to 400ppm NaCl before flushing out. Total marketable yield reductions (due to lowered fruit size) occur with 200ppm NaCl in the starting solution, with a permitted build up to 400ppm (Attenburrow and Waller,1980). Curiously fruit quality improves when the recirculating solution conductivity falls below 2mmhos under these circumstances. In neither the effluent nor the inorganic treatments for either experiment was the conductivity permitted to fall below this level. The sodium accumulation problem was avoided in the second experiment by flushing out every 10 days. In the first experiment the specifically noted reduction in fruit weight was not encountered, but again, the unusual circumstances of the termination of the experiment preclude any inference.

TableIII.4.3.4.a. Mains Water Quality, Selected Parameters.

Parameter	mg. L^{-1}, except where specified.
pH	7.57
Electrical Conductivity (mmhos).	1010
Ammonium-N	0.05
Nitrate-N	5.2
Soluble Phosphate (as P).	0.01
Potassium	9.4
Calcium	107
Magnesium	9.2
Sodium	100
Chloride	59
Sulphate (as SO_4'').	192
Zinc	0.1
Copper	0.01
Iron	0.03
Manganese	0.01

III.4.3.5. Calcium and Magnesium.

The high pH of the effluent indicates that most calcium and potassium will not be in solution. Discarding the settleable solids will cause the loss of these two elements from the supernatant which forms the basis of the recirculating solution. Furthermore, pig effluent has intrinsically low concentrations of both Ca and Mg (Taiganides and Hazen,1966).

The leaf margin chlorosis and necrosis observed in the first experiment, thought to be calcium deficiency, indicate that that element is limiting, notwithstanding the high Ca status in the local tap water (Table III.4.3.4.a.). Low plant levels of calcium are the cause of BER (Ward,1973), so intrinsic deficiency will cause this disorder. Other mechanisms are known to be involved, however. Pill, Lambeth and Hinckley (1978) suggest that BER may be caused by plant water stress due to either substrate water deficit, substrate osmotic potential, or changes in transpiration rate. They also point to high nitrogen fertilisation, especially high ammonium nitrogen as a proportion of total nitrogen. Both these latter conditions occur in the effluent treatments. There is still some conjecture on the details of the mechanisms involved in creating this disorder, but it is agreed that, as a cation, ammonium-N competes for uptake with metallic cations, notably calcium. Calcium is essential for cell wall generation, and low levels result in malformed fruits.

The high incidence of BER in the control plants in the first experiment is apparently contrary to this explanation. However, BER is also a function of moisture deficiency, and during the course of this experiment the electricity supply to the pumps failed three times, causing some wilting. This was greater for the control plants, which, being larger, were more susceptible to water stress.

In the second experiment the enforced aerated, and control treatements had significantly lower percentages of fruits with BER than the unaerated treatment. This indicates that aeration was sufficient to oxidise the ammonium-N, and sufficient calcium was available in the tap water to prevent absolute deficiency.

III.4.3.6. Potassium.

The symptoms of potassium deficiency were observed in the first experiment, and apparently rectified by the application of KNO_3. This strategy was employed from the outset in the second experiment, but K deficiency was still observed.

The tomato cultivar used in this trial is non commercial, and not greenback resistant. This condition is caused by excessive heat and light, and potassium deficiency (Kingham,1973). Assuming environmental factors to be equal, variation in the percentage occurrence of greenback may be attributed to low potassium levels. No significant differences were observed between any effluent runs, and the control levels were significantly lower. Table III.2.2.a. indicates a reasonable level of potassium, compared to the hydroponic recipes given by Cooper (1979), and Long Ashton (Hewitt,1966). The deficiency arises when potassium levels are considered in relationship to other plant nutrients.

III.4.4. Electrical Conductivity.

The use of electrical conductivity as a measure of nutrient status in recirculating hydroponics systems is very common. It has become so due to the understanding of crop nutrient requirements over the growing season (Schippers, 1980). In inorganic hydroponic nutrient solutions, more or less strict ratios between plant nutrients have been established. Two successful and popular compositions are given in Table III.2.2.a., demonstrating
these relationships. They appear to hold: Steiner (1980) in work on tomatoes and lettuce supports these ratios in observations made in ion uptake studies, for both cations and anions.

There has been some departure from the strict regulation of nutrient solution composition. In Cooper and Charlesworth's paper (1973) demonstrating comparable yields between NFT and conventionally grown tomatoes, precise control was kept over nutrient composition, to the extent of altering it weekly. The present practice is to reduce maintenance of solution composition wherever possible by using preformulated 'starter' solutions with 'replenisher' mixtures when

conductivity levels fall (Grower Books,1982). This must lead to some loss in efficiency of nutrient utilisation, as sufficient ions must be present to accomodate changes in specific ion requirements of the plant, depending on its developmental stage.

Fruiting plants, including tomatoes, require variation in the amounts of nitrogen and potassium in fruit formation. The K:N ratio changes from 1:1.25 at planting out, to 1:0.83 when the first two trusses are setting, and 1:0.42 with further truss development (Bedding,1973). The nutrient solution must be sufficiently flexible to accommodate increased demands on potassium at different stages (i.e. there must be deviations from the theoretical nutrient optima). If the deviations from these theoretical optima are not too great in the case of the effluent, then it is reasonable to use electrical conductivity as an index of nutrient status of the recirculating solution.

This approach is not without problems, however. The inorganic solution used here-'Libsol'- has sufficient potassium to meet the high K demand when it occurs. This is not the case with diluted effluent, which requires supplements. The aerated treatment gave a significantly lower yield than the control, and while some of the yield reduction may be attributable to BOD_5 and phenoxy compounds, nutrient limitations, other than potassium, must play a part.

Furthermore, the effluent, and to some extent the local tap water itself, have non-contributory ions present, notably Na, Cl, and SO_4, and in the case of the effluent, soluble organics. These make up a 'ghost' component in electrical conductivity measurements, reducing the levels of plant essential nutrients present in a solution of a given conductivity. Replenishing the solution adds to the problem, indicating a need to carry out frequent flushing of the system.

III.5. Conclusions.

The results suggest that there is not a clear case to be made for the use of anaerobically digested pig effluent as a medium for hydroponics, within the framework defined by this experimental trial. There are, however, indications that a modified slurry may be of use within this context. Enforced aeration considerably improved the overall yield in the second experiment, and ameliorated the problem of BER. Aeration demonstrates other benefits: removal of the suspended solids which render pH control difficult, and oxidation of ammonium nitrogen.

It is unclear whether the nutrient profile of the effluent is sufficiently balanced to allow the system to be controlled by the measurement of electrical conductivity alone, but this may only be established if the problems associated with the reduced condition of the effluent are removed.

APPENDIX IV COPPER.

IV.1.Introduction.

Copper is routinely added to the diet of pigs as a growth promoter. Additions of mineral copper give improved feed conversion ratios on identical diets. Wilson, Brigstocke and Cooke (1979) estimate that at the optimum level of copper addition to feed (224 ppm on a dry weight basis), a 6.5% improvement in liveweight gain is achieved.

Much of the added copper is voided by the animal, however. The worst estimates are that 95% of added copper is lost in this way. Botany Bay Farm adds copper at the present legal maximum level (200 ppm). If most of this is voided, the use of slurry in subsequent food production applications may give rise to problems in the form of excessive uptake by plants and animals. This has two main implications: toxicity effects for the immediate users of the slurry, or excessively high levels in the products destined for human consumption. It is desirable, therefore, to establish the fate of copper voided from the pigs.

This is achieved by determining the copper contents of both digested and undigested slurry from the farm in question, followed by an analysis of the oxidised effluent, both liquid and solid fractions to establish levels in the protein extract, and hydroponics medium. Finally, uptake by both tomatoes and fish will be determined.

The determinations are carried out following the spectrophotometric method (ADAS, 1981). For the slurry and tomatoes three replicates of each sample are taken, and four for the fish.

IV.2.1. The Slurry.

Table IV.2.1.a. shows the copper content of both digested and undigested slurry on a dry weight basis, together with values for both the liquid and solid products from the second aeration experiment. Although solids reduction is a feature of digestion, a change in copper concentration is not expected as the liquid volume remains the same. What is suprising is the low copper levels in the effluent. If copper additions are maintained at 200ppm and, as Wilson, Brigstocke and Cooke (op. cit.) suggest, 95% is voided, then the levels in both the digested and undigested slurry would be expected to be higher.

Of the two products from the aeration process, the copper is being concentrated in the solid fraction. McGill et al. (1975), in a study of the distribution of copper in raw pig slurry, found that most of the copper is associated with the solids. While the method of preparation was crude (the whole slurry being centrifuged at 2500rpm, and the supernatant filtered through a 0.45μm millipore, the results demonstrated that the bulk of the copper was associated with the centrifuged solids, with a significant proportion being found in the suspended solid fraction. Almost none was present in the filtered solution. Moore et al.(1969) have suggested that copper forms a protein complex in pigs, causing this association between solids and copper, which is originally introduced as $CuSO_4$ in the diet. As the solid fraction of the slurry is being taken into the biofilm, these results are consistent with this complexing mechanism, and suggest that digestion does not change the association of copper with organic nitrogen.

IV.2.2. Copper In Tomatoes.

The nutrient solutions employed in the effluent treatments would not be expected to give very high fruit copper concentrations. The copper levels, although elevated, are relatively low, compared to concentrations found in soils which are considered to offer no risk. Davies (1977) reports on a

Table IV.2.1.a. Copper Concentrations in Digested and Undigested Slurry, and Protein Extract from the Second Aeration Experiment, with Supernatant, aerated liquid.

Parameter	Copper
Undigested Whole Slurry(ppm)	61.49(5.17)
Digested Whole Slurry (ppm)	60.82(4.25)
Protein Extract (mg. Kg^{-1}, dry Weight)	1002(364)
Supernatant Liquid (mg L^{-1})	11.32(0.68)

Figures in Brackets are Standard Errors of the Mean.

Table IV.2.2.a. Copper Levels in Nutrient Solutions, and in Fruits, Shoots, and Roots of the Tomato Plants, 2nd Experiment.

	Libsol	'K-Rel'	'DOPE-K'
Copper in Starter Solutions ($mg.L^{-1}$)	0.07	1.07	1.32
Copper in Make-up Solutions ($mg.L^{-1}$)	0.07	0.46	0.99
Copper in Fruits ($mg.Kg^{-1}$)	9.86(1.83)	12.13(2.17)	9.70(0.34)
Copper in Shoots ($mg.Kg^{-1}$)	7.71(2.24)	7.79(0.63)	15.09(3.01)
Copper in Roots ($mg.Kg^{-1}$)	27.60(7.03)	84.40(21.75)	158.73(53.55)

Numbers in brackets are standard errors of the mean.

Values for plants are on a dry weight basis.

Values for Copper in Solution are taken from Manufacturer's Specifications, or ADAS results.

relatively low uptake by lettuce over a range of soil copper concentrations. The foliar values given are 4.9, 6.8, 8.6, and 9.3 mg Kg^{-1} dry matter for EDTA extractable (i.e. plant available) copper levels of 6, 62, 131, and 197 mg L^{-1} respectively in a soil amended with inorganic copper. Davis (1981) reports that uptake levels for tomato in a sewage sludge contaminated soil containing 113 mg Kg^{-1} copper (total, or EDTA extracted is not specified) are 11.5 and 8.0 mg Kg^{-1} dry matter in leaves and fruits.

It is not strictly accurate to consider EDTA extractable copper (copper which is not bound to minerals or stable humic complexes) as analagous to the copper in the effluent NFT solutions, as exchangeable copper is not present. However, this is a reasonable approximation, and under this model, anticipated copper levels in the plants will not be high.

Table IV.2.2.a. shows the copper levels in tomato fruits, leaves and roots for the Libsol, effluent plus potassium, and effluent with added nutrients, treatments, together with the copper levels in the respective nutrient solutions.

The copper in the NFT solution is almost certainly not in the mineral form. Graham (1981) indicates that in both soil and solution cultures, copper is almost entirely complexed either by root exudates, or organic ligands. The number of organic species with which copper can chelate is extensive. Stevenson and Fitch (1981) mention aliphatic acids, amino acids, phenolics, peptides and proteins, polysaccharides, and humic and fulvic acids. Each of these have a varying stability, depending on solution ionic concentration and pH. At the level of resolution where only total copper in the nutrient solutions is measured, it is sufficient to assume that the copper is chelated, and plant available.

Copper is probably not, however, absorbed by the plant as a chelate. Although the chelate is the predominant copper form in solution, the ligand is lost prior to uptake (Graham, op. cit.)

Plant copper concentration is not dependent solely on solution concentration. Jarvis (1981) implicates other nutrients (N, P, Fe, and Zn) in copper uptake. High nitrogen concentration is especially important, as it may bring about increased absorbtion and transport within the plant at

Table IV.2.3.a. Copper Levels in Diets and Fish Culled at the End of the Second Fish Nutrition Experiment.

	Control	10%	20%
Diets (mg Kg^{-1})	8.67(2.22)	55.10(2.91)	189.93(15.27)
Carcass(mg Kg^{-1}).	76.65(4.70)	86.70(5.70)	113.01(12.67)

Numbers in brackets are standard errors of the means.

All values are on a dry weight basis.

luxury copper levels in solution culture. While the nitrogen levels in the NFT solutions were perforce different, none deviated excessively from the Libsol norm, and no high concentrations were found in the plant tops.

The high levels of copper found in the roots is expected. The root concentrations are generally higher than those in the shoots. This difference is enhanced with increased availability of copper (Loneragan,1981).

IV.2.3. Fish.

In fish feed formulation it is assumed that copper is an essential trace element, a belief held entirely due to the need for copper by warm blooded animals. Very little work has been done on the subject, and mineral mixtures usually ape the formulations used for mammals (Jauncey, 1982). It is clear, however, that high levels of mineral copper are toxic to fish: Wong et al.(1977) observed that mirror carp (Cyprinus carpio) subjected to 10 ppm $CuSO_4$ survive for only 50 minutes. The recommended safe solution level of copper for Cyprinids is 0.02 ppm Cu (Anglia Water Authority recommendations). Chelated copper in the diet is not neccessarily as toxic, and may not even be available in the neutral to slightly alkaline environment of the fish gut. Stickney et al. (1974) discovered lower copper levels in 11 species of estuarine fish, compared to the levels found in the major food organisms of the fish, indicating that ingestion is not synonymous with uptake.

Table IV.2.3.a. shows the levels of copper in the three experimental diets, and that in sample fish culled at the end of the second experiment.

The diets, as would be expected, show increasing copper levels with substitution, which is reflected in the copper contents of the fish at the end of the experiment. The values are elevated, due to quality problems. The second fish experiment was carried out in a new building, with new piping, which proved to be made of copper. The uniformly low feed conversion ratios experienced in this experiment caused a search for the cause of the problem. An examination of the water by the Anglia Water Authority showed that solution copper levels were 0.2ppm Cu., a factor of 10 above the recommended upper limit. This resulted in abnormally high

copper levels in the fish, even in the control diet. In order to isolate the copper contribution due to the dietary components, a regression analysis was carried out. The derived expression, Y=0.21X+75.26, where X is the copper in the diet, and Y is the copper in the fish, indicates that for every unit of copper in the diet ingested, only 0.21 (0.06) units (standard error) is taken up by the fish. This is in accord with other work on heavy metal uptake by fish. Singh and Ferns (1978) found no significant increase in copper concentrations in rainbow trout (Salmo gardnieri) fed activated sludge containing 176 ppm copper, although interestingly found significant increases in chromium, iron, nickel and lead. Similarly Tacon and Ferns (1976), and Tacon (1979) found no significant increase in copper in the carcasses of rainbow trout also fed on activated sludge. The low uptake levels was rather baldly attributed to the unavailable form of the copper.

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GLOSSARY.

A.D.	Anaerobic Digestion. The technique of subjecting wet organic materials to oxygen-free conditions, in order to capture methane produced by naturally occurring methanogenic bacteria.-see Chapter 1 and Appendix 1.
BOD	Biochemical Oxygen Demand. A measure of the oxygen removed from a diluted sample of the liquid under test over a prescribed incubation period, normally five days (hence BOD_5). A measure of the polluting potential of the liquid, and a guide to the 'methane potential' of a liquid.
COD	Chemical Oxygen Demand. A measure of the oxygen absorbed by a sample from boiling acidic dichromate solution. It is a 'belt and braces' measurement of all reduced compounds in the liquid, and removes only slowly available organic compounds, and reduced inorganic species. As such, it will overestimate the polluting, and methane yielding potential of a liquid.
NFT	Nutrient Film Technique. A hydroponic method of plant production in which the roots of the growing plants are contained unsupported in a channel through which a thin film of the nutrient solution is flowing.-See Chapter 8.
RBC	Rotating Biological Contactor. A type of aeration system in which the active microbial population grow on corrugated plastic discs which are sequentially immersed in the liquid being treated, and the air, by rotating the discs about their centres. See Chapter 4.
TS	Total Solids. The weight of solids in a liquid sample, as determined by the dessication of the sample in an oven set at 105°C for 18 hours. A crude guide to the polluting potential of the liquid.
VS	Volatile Solids. Those solids which are lost (i.e. oxidised) when a sample of solids dried by the total solids method are subjected to a temperature of 550°C for 18 hours. A slightly less crude index of the polluting potential of the solids in a liquid, compared to total solids.

NOTE.

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